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Estoques de C e N em Cerradão sob diferentes usos e suas dinâmicas no solo

C and N stocks in Brazilian woodland savanna (Cerradão) under different land uses, and their dynamics in the soil

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“ Na vida, não existe nada a temer, mas a entender. ”

Marie Curie

RESUMO

BRITO, Gisele Silveira. **Estoques de C e N em Cerradão sob diferentes usos e suas dinâmicas no solo**. 2018. 115 f. Tese (Doutorado em Ecologia) – Instituto de Biociências, Universidade de São Paulo, São Paulo, 2018.

A substituição da vegetação nativa por pastagem e silvicultura pode resultar em mudanças nas concentrações, estoques e dinâmica do carbono e nitrogênio. O objetivo do trabalho foi analisar o impacto da conversão de cerradão em pastagens de braquiária e plantações de eucalipto na concentração de variáveis da fração lábil do carbono orgânico do solo (SOC) e na respiração edáfica basal, nas concentrações das variáveis de nitrogênio e no seu potencial de mineralização (PMN), para os primeiros 200 cm de profundidade do solo. Foi ainda avaliada a variação sazonal na variabilidade de C e N no solo. Investigamos ainda os estoques de C e N abaixo do solo (até 50 cm de profundidade) e os estoques de C acima do solo. Nossas amostragens foram realizadas em três áreas de pesquisa, numa região originalmente coberta por cerradão. Cada área de pesquisa era constituída por um sítio controle (Cerrado) e dois usos das terras (pastagem, eucalipto). Amostras de solo foram coletadas nas profundidades de 0-10, 10-30, 30-50, 50-100 e 100-200 cm durante as estações seca e chuvosa, para as análises das frações de C e N; para o cálculo dos estoques de C e N, foram utilizadas somente as três primeiras profundidades. Coletamos serapilheira e biomassa de herbáceas + arbustos, e tiramos medidas das árvores para o cálculo do estoque de C acima do solo. Nossos resultados mostraram uma redução de aproximadamente 50% da biomassa microbiana (MBC) e da taxa MBC:SOC para pastagens e plantios de eucalipto, indicando menor estabilidade do carbono orgânico do solo. Foram registrados decréscimos para o carbono orgânico dissolvido (DOC) e para as taxas DOC:SOC, além de aumentos para o quociente metabólico em ambos os usos, até 30 ou 50 cm de profundidade. O efeito do uso das terras nas variáveis lábeis do C orgânico do solo foi mais acentuado na estação seca. As formas inorgânicas de nitrogênio (NH_4-N e NO_3-N) e PMN foram significativamente afetados pela conversão do uso da terra, com decréscimos em ambos os usos em comparação ao Cerrado nativo, sendo menores os valores encontrados no Eucalipto. A atividade da urese também decresceu com a mudança de uso. Todas as variáveis de N diminuíram com a profundidade de 10 a 50 cm. A conversão de uso também resultou em perdas nos estoques de C e N. O estoque total de C em pastagens reduziu em 53% e nas plantações de eucalipto ocorreu aumento de 20%. Na pastagem, foi registrada uma redução de 94% no estoque da biomassa aérea, em comparação com o Cerrado, enquanto que as plantações de eucalipto apresentaram um aumento de 80%; abaixo do solo, as pastagens tiveram reduções de 19% e 25% nos estoques totais de C e N. As plantações de eucalipto tiveram redução de 23% para o estoque total de C abaixo do solo e de 19% para o estoque de N (abaixo do solo). Ambos os usos da terra tiveram perdas totais semelhantes para os estoques de C e N abaixo do solo, mais significativos na camada de 10-30 cm. Nossos resultados demonstraram impactos negativos da conversão do cerrado em pastagens e plantações de eucalipto para a variáveis carbono e nitrogênio, com redução da capacidade de estoque no solo, comunidades microbianas menos eficientes e redução da capacidade de mineralização.

Palavras-chave: Cerrado, ciclo do carbono, ciclo do nitrogênio, estoque de carbono, estoque de nitrogênio, silvicultura de eucalipto, pastagem de *Urochloa*, mudança do uso da terra.

ABSTRACT

BRITO, Gisele Silveira. **C and N stocks in Brazilian woodland savanna (Cerradão) under different land uses and their dynamics in the soil**. 2018. 115 f. Tese (Doutorado em Ecologia) – Instituto de Biociências, Universidade de São Paulo, São Paulo, 2018.

The replacement of the native vegetation by pastures and silviculture can result in clear changes on the carbon and nitrogen pools and stocks. We aimed to assess the impact of the woodland cerrado (*cerradão*) conversion into pastures and *Eucalyptus* plantations on the soil organic carbon (SOC) labile pools, on the concentrations of N variables and on the potential for nitrogen mineralization (PMN) in the first 200 cm of the soil profile. We also assessed the seasonal variation on the overall variability in soil C and N pools. Finally, we investigated the C and N stocks belowground (up to 50 cm depth) and C stocks aboveground. We had three sampling sites in a region originally covered by *cerradão* physiognomy. Each sampling included a control area (Cerrado) and two land uses (Pasture, *Eucalyptus*). Soil samples were taken at 0-10, 10-30, 30-50, 50-100 and 100-200 cm depths during the dry and wet climate seasons, from which we used the first three depths for stocks calculation. We also collected litter and herbs+shrubs biomass, and measured the tree biomass for C stocks calculation. Our results showed ~50% reduction of the microbial biomass (MBC) and MBC:SOC for pastures and *Eucalyptus* plantations, indicating lower SOC stability. Reduction in the dissolved organic carbon (DOC) and DOC:SOC, and increment on the metabolic quotient were also registered for both land uses along the soil profile up to 30 or 50 cm depth. Land use effect on SOC labile pools was more marked in the dry season. The inorganic forms of nitrogen (NH_4-N and NO_3-N) and PMN were significantly affected by land use conversion, with decreases in both land uses compared to native Cerrado, with lower values found in *Eucalyptus*. Urease activity also decreased with land conversion. The N variables all decreased with depth from 10 to 50 cm depth. Land conversion also resulted in C and N stock losses, and in redistribution among the system compartments. Pastures showed 53% less overall C stock, and *Eucalyptus* plantations had 20% more. Aboveground, C was 94% reduced in pastures compared to Cerrado, and 80% increased in *Eucalyptus* plantations; belowground, pastures had 19% and 25% reductions in the overall C and N stocks respectively. *Eucalyptus* plantations had 23% decrease in C and 19% in N stocks. Both land uses had similar overall losses of C and N belowground, which were higher at the 10-30 cm layer. Our results show negative impacts on carbon and nitrogen pools, cycling processes and stocks due to Cerrado conversion to pastures and *Eucalyptus* plantations, with reduced storage capacity, less efficient microbial communities and power potential for mineralization.

Keywords: Cerrado, *Eucalyptus* plantation, *Urochloa* pasture, land use change, carbon cycling, nitrogen cycling, carbon stock, nitrogen stock.

SUMMARY

General Introduction 13

References 17

Chapter 1. Soil labile organic carbon pools of Brazilian woodland savanna (cerradão) soils under different land uses 23

Abstract 23

Introduction 24

Materials and Methods 26

Results 30

Discussion 37

Conclusion 42

References 44

Supplementary Material 54

Chapter 2. The conversion of Brazilian woodland savanna (cerradão) to pasture and Eucalyptus plantations reduces soil nitrogen stocks and mineralization 56

Abstract 56

Introduction 57

Materials and methods 60

Results 62

Discussion 69

Conclusion 73

References 74

Supplementary material 80

Chapter 3. Changes in carbon and nitrogen stocks after conversion of Brazilian woodland savanna (Cerradão) to pasture and silviculture 82

Abstract 82

Introduction 83

Materials and Methods 86

Results 89

Discussion 99

Conclusion 101

References 102

Supplementary Material 113

General Conclusion 115

GENERAL INTRODUCTION

The conversion of native vegetation into agriculture, silviculture and pastures can undermine the capacity of the natural terrestrial environments to sustain important ecosystem services such as soil fertility, nutrient cycling and climate regulation (Foley *et al*., 2005; Foley, 2017). Approximately 80% of ecosystem services may be associated to soil functions (Lal, 2001), in processes that depend on the natural biodiversity, such as the carbon and nitrogen biogeochemical cycles. Terrestrial ecosystems are a vital part of the global carbon cycle due to their carbon storage capacity (Houghton et al., 2009), where vegetation and soil act as carbon sinks, storing the CO_2 that is absorbed through photosynthesis. Soil is the terrestrial compartment that contains the largest C stock in the planet (Lal et al., 2003; Jazen, 2014; Maillard et al., 2017) having a great potential to mitigate or aggravate the global climate change even with small shifts in the C pools (Piao et al., 2012; Jazen, 2014; Maillard et al., 2017). Soil C storage depends, among other things, on the N availability, as nitrogen is a limiting nutrient for plant growth (Vitousek and Howarth, 1991; Aerts and Chapin, 2000; Wang et al., 2010). In that way, C and N cycles are mutually connected, and the kind of C compounds in litter controls decomposition and N availability to plants, which in turn controls the rate of biomass production (Pastor and Post 1986).

The C and N cycles mainly depend on plant-soil interactions and responses (Lavelle et al. 2005), and the conversion of natural ecosystems to agroecosystems often result in nutrients stock losses and greenhouse gas emissions, due to changes in the soil organic matter arrangement and decomposition process (Lima et al. 2000, DeGryze et al. 2004). Agricultural practices provide specific organic and inorganic inputs that are normally fragmented through bacterial processes that can release significant amounts of CO_2, CH_4 and N_2O to the atmosphere (IPCC 2014). The sector of agriculture, forestry and other land uses was considered the third leading source of C emissions from 1750 to 2011, and was responsible for 25% of greenhouse gases emission in 2010 (IPCC, 2014; Smith et al., 2014). In order to mitigate the impact of land use conversion on the C and N biogeochemical cycles it is indispensable to fully understand and quantify regional and local dynamics of these cycles individually, and also the relationship between them, as the real impact will depend on specific environmental features and on the characteristics of the new land use.

The environmental impact of agricultural expansion has risen side by side to global demand for food, fiber and fuel. According to Tilman *et al*. (2011), numerical valuations show that the environmental impact for achieving future population demand for food will depend on how global agriculture expands. The increasing change on land use and land cover (LULC) in tropical regions is a vital part on meeting global demand for agricultural commodities, however, it leads to extensive environmental impact (Guillaume *et al*., 2018). Tropical lands – especially in South America and the sub-Saharan Africa – are the most intensively converted regions in the planet for the last decades (Gibbs *et al.*, 2010; Phalan *et al.*, 2013), mostly due to the countries non-compliance with environmental legislation, low production costs, substantial potential to achieve increased harvest and land availability (Alexandratos and Haen, 1995; Gibbs *et al*., 2010, Guillaume *et al*., 2018). One of the main land uses in tropical regions is the conversion of forests into agricultural lands (Don *et al*. 2011). Tropical forests store almost 60% of the total C of all forests around the world (Batjes, 1996; Durigan, 2013). Land conversion in the tropics has resulted in climatic alterations, biodiversity losses and changes in soil fertility (Asner *et al*. 2009, Durigan 2013), which made this activity responsible for 12-20% of the global anthropogenic greenhouse gas emissions (IPCC, 2007; Don *et al*., 2011).

In this scenario, Brazil has emerged as the world's second major agricultural exporter, and is likely that, by 2050, the country will supply 40% of the global food demand (OECD/Food and Agriculture Organization of the United Nations, 2015; Sousa-Neto *et al*., 2018.). However, the agricultural sector achievement is connected to great impacts on the Brazilian ecosystems, at present particularly the Brazilian savanna (Cerrado) and the Amazon forest (Martinelli et al. 2010). Agriculture together with livestock farming are responsible for 30% of the total emission of greenhouse gases in the country (SEEG, 2017). Brazil is currently one of the five nations with the highest CO_2 emissions in the planet (Baccini *et al*. 2012; Matthews *et al*. 2014; Camil *et al*., 2017).

The Cerrado biome has an area of almost 2 million km^2 and is placed in the central portion of the Brazilian territory (Ribeiro and Walter, 1998). The vegetation is formed by several vegetation types, from savanna to forest formations (Eiten, 1982; Redford and Fonseca, 1986), which shelter a huge species richness and high proportion of plant endemism (Klink and Machado, 2005). Thanks to its high biodiversity and degradation state, Cerrado is considered one of the world's hotspots (Myers *et al*., 2000). Currently, almost half of the Cerrado domain has been converted to other land uses (Machado *et al*., 2004; Lapola *et al.*, 2014) such as *Eucalyptus* spp. forestry and brachiaria (*Urochloa* spp.) pastures, which are common land uses in the Southeastern part of the biome (Durigan *et al*. 2007). Deforestation, soil liming and fertilization, machine traffic and cattle trampling are part of the establishment and management procedures associated to forestry and pastures in the Cerrado, which frequently result in changes on litter composition and quantity, meso and microbiota, soil density, pH and nutrient availability (Frazão *et al*. 2010). Soil C and N stocks and dynamics, which mainly depend on the soil organic matter decomposition process, may respond to these new environmental conditions by reducing storage capacity, increasing greenhouse gases emission to the atmosphere, and even by contaminating the water resources.

According to Wang *et al*. (2010), 19% of the overall C stock of the terrestrial biosphere is stored in living plants, 4% in litter and 77% in the soil organic matter, while the overall N stock is mostly in the soil (94%), being only 5% stored in living plants and 1% in litter. Carbon and nitrogen fixed by photosynthesis in plant tissues are stored in the aboveground biomass and later pass to the soil compartment through litter decomposition. In the soil, C and N transit among different compartments, with regulatory variables constituting soil C and N dynamics (Srivastava *et al.*, 2017). Both elements can be found in the soil in organic and inorganic forms (Pulronik et al. 2009; Cameron and Posner, 1979). Soil organic carbon (SOC) and soil organic nitrogen (SON) are contained in plant roots, soil biota and in soil free organic matter (SOM). The material that is in transition between fresh plant residues and stabilized organic matter is the labile fraction of SOM, and it is very important for the maintenance of soil fertility, being less resistant to microbial decomposition (Haynes 2005). The labile C fraction of SOM is classified as *passive labile C*, represented by roots exudates and other dissolved organic matter; and as *active labile C*, represented by the microbial biomass. Before SON can be taken by plants, it has to be converted to inorganic forms, mostly ammonium (NH_4^+) and nitrate (NO_3^-), which can be found soluble in the soil solution, and represent the main N forms absorbed by plants (Cameron and Posner, 1979).

Soil microbial biomass is responsible for SOM decomposition, that produces the largest flux of nutrients on Earth. During decomposition, part of carbon is mineralized (process also known as soil respiration) and released to the atmosphere as CO_2, while another part is reincorporated into microbial biomass, making it an important C storage compartment (Jenkinson and Ladd, 1981; MEA, 2005; Pulronik et al., 2009; Srivastava *et al*., 2017). As a by-product of SOM decomposition we have the mineralization process, which consists in transforming SON into inorganic forms. Conversion of native vegetation into agricultural activities usually modify the soil properties, litter quality and quantity, and also the microclimate, affecting SOM structure and the microorganisms community (Brye et al. 2002; DeGryze et al. 2004; Anderson et al. 2010). Microbial biomass depletions and metabolic activity variations alter the way SOM decomposes and disturbs the C and N cycling, often reducing soil storage capacity and increasing CO_2 emissions to the atmosphere (Giller et al. 1998; Aduan et al., 2003). Interactions between C and N cycles have an important part in determining the long-term evolution of plant, litter, and soil organic matter compartments (Vitousek and Howarth, 1991). C and N storage is then a promising option for mitigating global climate change (Lal, 2003; Thornton et al., 2007).

The assessment of labile SOC pools, inorganic forms of N, and microbial biomass activity indicators, such as soil basal respiration, potentially mineralizable nitrogen (PMN) rates, and urease activity can provide essential information on soil C and N dynamics. These soil biochemical parameters are highly sensitive indicators of environmental changes caused by LULC alterations (Paul *et al*., 1999; Araújo and Monteiro, 2007; Rocha Junior *et al*., 2013). Variations in soil C and N stocks and dynamics due to land use changes offer vital information on soil ecosystem services, such as fertility and C storage capacity, enabling the land use planning and the recovery of degraded areas. Considering these compartments lability and high sensitivity to environmental changes, results can give an effective early warning on the deterioration of soil quality (Powlson et al., 1987; Zilli et al., 2003; Wardle, 1992).

In this context, we approached the C and N pools and stocks in the woodland Cerrado ecosystem (*cerradão*) and af ter being converted to *Eucalyptus* plantation and brachiaria pasture in the soil (belowground), and we also assessed the C stocks of the aboveground biomass.

Chapter 1 deals with the impact of LULC change on soil organic carbon (SOC) labile pools (microbial biomass carbon, dissolved organic carbon, microbial quotient, soil basal respiration, metabolic quotient) associated to soil depth and climate seasonality. Our results showed reductions of the labile C pools (up to 50 cm depth), and also pointed to microbial activity alterations in pastures and *Eucalyptus* plantations, leading to lower efficiency on litter decomposition. We also reported reductions of the rate between microbial biomass carbon and total soil organic carbon, indicating that less C is being converted as microbial biomass, indicating lower SOC stability for the target land uses.

Our results reveled overall negative effects on the C cycle after the conversion of native Cerrado to pastures and *Eucalyptus* plantations.

In Chapter 2 we assessed the impact of *cerradão* conversion to pastures and *Eucalyptus* silviculture on the concentrations and stocks of total nitrogen (N), inorganic forms of N (NH_4-N and NO_3–N), and on the potential for nitrogen mineralization through the rates of potentially mineralizable nitrogen (PMN) and soil urease activity. The NH_4-N, NO_3-N and PMN were significantly affected by LULC change, with overall reductions in both land uses compared to native Cerrado, especially in *Eucalyptus* plantations. Reductions were detected up to 50 cm depth in soil. The inorganic N stock decreased in pastures, while NH_4-N stock in *Eucalyptus* plantations decreased and NO_3-N stock increased. Both land uses reduced PMN rates per unit area. Results also pointed to negative effects on the N cycle due to Cerrado conversion into pastures and *Eucalyptus* plantations.

In Chapter 3 we aimed to assess the effect of LULC change on C (above and belowground) and N (belowground) stocks, distribution, and overall balance after the Cerrado conversion to brachiaria pastures and *Eucalyptus* silviculture. Results show C and N stock losses and redistribution among the system compartments in both land uses. Carbon was mostly stored belowground in pastures, and aboveground in *Eucalyptus* plantations, while Cerradão showed a balanced distribution. Losses in both above and belowground stocks were verified for pastures while in *Eucalyptus* plantations, the aboveground C stock increased and the belowground C and N stocks reduced. Results point to lower C and N storage capacity, especially in the soil, in both target land uses.

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CHAPTER 1

Soil labile organic carbon pools of Brazilian woodland savanna (*cerradão*) soils under different land uses

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Abstract

Conversion of native vegetation to different agricultural uses alters the carbon cycling, usually reducing soil storage capacity and increasing CO_2 emissions to the atmosphere. We assessed the impact of land use/land cover (LULC) on soil organic carbon (SOC) labile pools of Brazilian savanna (Cerrado) soils under pasture and *Eucalyptus* plantation, and determined alterations related to soil depth and climate seasonality. In a region originally covered by *cerradão* physiognomy we had three sampling sites composed by a control area (Cerrado) and two land uses (Pasture, *Eucalyptus*). Soil samples were taken at 0-10, 10-30, 30-50, 50-100 and 100-200 cm depths during the dry and wet climate seasons. We quantified the dissolved organic carbon (DOC) and the microbial biomass carbon (MBC), calculated the microbial quotient (MBC:SOC) and the DOC:SOC ratio, and evaluated the soil basal respiration (SBR) and the metabolic quotient (qCO_2). Our results showed MBC, MBC:SOC, DOC, DOC:SOC reductions, and qCO_2 increases along the soil profile up to 30 and 50 cm depth, for Pasture and *Eucalyptus*. MBC stocks decreased by ~50% in both land uses in the dry season, while DOC stocks decreased by 11% in *Eucalyptus* and 15% in pastures. MBC and MBC:SOC reductions indicate lower SOC stability. Higher SBR and qCO_2 rates are related to a microbial community under stress. The target land uses showed higher MBC and DOC values at 30-50 cm depth, but Cerrado higher values happened at soil surface. Land use effect on SOC labile pools was more evident in the dry season (higher DOC concentrations and SBR rates); contrarily, higher MBC stocks occurred in the wet season. The qCO_2 was also higher in the dry season indicating higher microbial activity (associated to DOC stocks) and/or a lower efficiency on decomposing litter, especially in Pastures and *Eucalyptus*. Concluding, *cerradão* conversion to pastures and *Eucalyptus* plantations under common management practices negatively affected SOC labile pools.

Key words: Cerrado, land use change, carbon cycling, *Eucalyptus* plantation, *Urochloa* pasture, dissolved organic carbon, microbial biomass carbon, metabolic quotient, microbial quotient.

Introduction

Carbon cycling can be highly influenced by land cover changes (Lavelle *et al*., 2005), as it depends on multiple interactions of different ecosystem compartments, especially between soil and vegetation (Brye *et al*., 2002; Arora and Boer, 2010). Conversion of native vegetation into agriculture, for example, can modify soil physical and chemical properties, litter amounts and composition, and the microclimate, affecting not only the quantity and quality of soil organic matter (SOM), but also the microorganism community, a vital control factor of the C cycling (Burket and Dick, 1998; Del Galdo *et al*., 2003; DeGryze *et al*., 2004; Anderson *et al*., 2010). Depending on the converted land use and features of the original ecosystem, soil can be either a sink or a source of carbon to the atmosphere (Eglin *et al*., 2010), being able to influence the global climate change even with small shifts in C pools (Janzen, 2004; Piao *et al*., 2012; Maillard *et al*., 2017). The current scenario of increasing global demand for food, fiber and fuel, and the associated greenhouse gas emissions makes the understanding and quantification of the impacts of new agricultural lands on the C cycling and storage a critical matter (Houghton, 2012).

The ultimate target for agriculture expansion are the tropics, especially South America and the sub-Saharan Africa (Phalan *et al*., 2013), where forest-rich developing countries where compliance with environmental legislation and production costs are low, such as Brazil, stand out (Alexandratos and Haen, 1995; Gibbs *et al*., 2010). Brazil has emerged as a leading agricultural producer, with increasing greenhouse gas emissions in the agricultural sector – 20% increase from 2000 to 2012 (MCTI, 2013) – coming especially from the soil compartment (Sousa-Neto *et al*., 2018). The Amazon and Cerrado biomes are the most transformed in Brazil, being responsible for most of the total gas emissions related to land use changes (54% from 1990 to 2014) (Lapola *et al*., 2013; Sousa-Neto *et al*., 2018).

The complex of grasslands, savannas and dry woodlands that compound the Cerrado biome takes over 23% of the Brazilian territory and is the world's richest savanna regarding plant species (Oliveira-Filho and Ratter, 2002; Forzza *et al*., 2012). That high biodiversity with numerous endemic flora species together with great threat takes Cerrado as a biodiversity hotspot (Myers *et al*., 2000; Klink and Machado, 2005). *Eucalyptus* sp. forestry and livestock pastures of exotic grasses are two of the most common land uses in the Cerrado biome (Durigan *et al*. 2007), especially in its Southeastern part, and are related to practices such as deforestation, fertilization (as Cerrado soils are acid and poor in available nutrients, Lopes and Cox 1977), and machine traffic, besides cattle trampling. These practices often result in alterations of litter quantity and composition, soil density and pH, meso and microbiota, and nutrients availability (Frazão *et al*. 2010). Soil C stocks and dynamics, which mainly depend on the SOM decomposition, can respond to these new environmental conditions by reducing storage capacity and increasing CO_2 emissions to the atmosphere.

Degraded pastures in the Cerrado biome have revealed significant soil organic carbon (SOC) losses (Silva *et al*. 2004, Maia *et al*. 2009; Carvalho *et al*. 2010; Salton *et al*. 2011). Despite the difficulty and subjectivity in defining a pasture degradation scale (Maia *et al*. 2010; Carvalho *et al*. 2014) it has been suggested that at least 36 million ha of Cerrado pastures are under some degree of degradation (Klink and Machado 2005; Costa *et al*. 2006). Management procedures, soil type, forage species, microclimatic conditions and temporal variations (seasonality), which reflect on the pasture productivity degree (Carvalho *et al*. 2014), can be determinants for soil C storage capacity in the Cerrado areas. Pastures with high biomass production and appropriate management practices, on the other hand, have shown to be potential C sinks in the Cerrado biome (Corazza *et al*. 1999; Lilienfein *et al*. 2003; Maia *et al*. 2009, Frazão *et al*. 2010). Studies on *Eucalyptus* plantations in the Cerrado have also reported ambiguous results – which can be associated to differences in soil type, fertilization practices, microclimatic conditions, vegetation age and seasonality – reporting SOC increases (Neufeldt 2002; Maquere 2004), substantial SOC losses (Zinn *et al*. 2002; Montero 2008) or no significant differences (Lepsch 1980), when compared to the native vegetation.

A way to enlighten the discussion on the effects of native Cerrado conversion into pastures and *Eucalyptus* plantations on soil C storage capacity is to assess the labile fraction of SOC, mostly represented by the dissolved organic carbon (DOC) and the microbial biomass carbon (MBC), which are associated to soil C dynamics. It is widely known that the labile fraction of soil organic carbon (SOC) is a very sensitive compartment to environmental alterations related to land use changes, due to its short residence time in the soil and rapid cycling (DeGryze *et al*. 2004; Haynes 2005; Yang *et al*. 2010; Geraei *et al*. 2016). For the Cerrado biome there is still a research gap on the knowledge about the quantification of the labile fraction of SOC. Most studies addressing the labile pools on livestock pastures and *Eucalyptus* plantations are mainly focused on the active labile C (microbial biomass carbon, MBC), reporting considerable decreases after land use conversion (Alvarenga *et al*. 1999; Araújo *et al*. 2007; Rangel and Silva 2007; Kaschuk *et al*. 2010; Ribeiro *et al*. 2010; Oliveira *et al*. 2016; Vinhal-Freitas *et al*. 2017). A small number of these studies approached a relationship to functions of C dynamics, such as correlations between C stocks and indicators of microbial activity. Still, assessment of the SOC labile pools alterations due to land use changes in Cerrado areas can provide an effective early warning on the deterioration of soil quality and C storage capacity (Powlson *et al*. 1987; Wardle 1992; Zilli *et al*. 2003), enabling a better land use planning and the recovery of degraded areas.

The objective of this study was to assess these questions: Is there any effect of land use/ land cover (LULC) on SOC labile pools of Cerrado soils under planted pastures and *Eucalyptus* plantations? If so, do the effects relate to soil depth and climate seasonality? How would the soil labile carbon pools affect nutrient cycling? Therefore, we compared in three research sites, each one including fragments of native Cerrado and areas converted into livestock *Urochloa* pastures and *Eucalyptus* plantations, SOC labile pools – by assessing the dissolved organic carbon (DOC) and the microbial biomass carbon (MBC) concentrations, by calculating the microbial quotient (MBC:SOC) and DOC/SOC ratio, by measuring the soil basal respiration (SBR) rates and also calculating the metabolic quotient (qCO_2). Considering that the Cerrado usually has deep soils and deeply rooted vegetation (Castro and Kauffman 1998), we also investigated the effect of LULC throughout the first 2 meters of the soil profile. In addition, we investigated the influence of climate season on SOC labile pools.

Materials and Methods

Study area

This study was conducted in the Southeastern portion of the Cerrado biome, in São Paulo state, Brazil, at Pirassununga and Bauru municipalities (Supplementary Material, Table S1) (Figure 1). Remaining native vegetation in these areas is restricted to numerous small fragments, due to intensive anthropic activities such as agriculture, forestry and cattle raising (Durigan 2006). The local climate is Koeppen’s Cwa (Pirassununga) and Aw (Bauru), both warm, humid, with wet summers and dry winters. Annual average rainfall in both locations varies between 1300 mm (Bauru weather station; 22º19’ S – 49º02’ W; 1945-2001 rainfall series) and 1420 mm (Pirassununga weather station; 21º58’ S – 47º28’ W; 1954-1999), being 75% of it concentrated in the summer (October to March) (DAEE, Department of Water and Electric Energy of São Paulo State). We had three research sites: Pirassununga (Pirassununga municipality), Santa Terezinha and Jardim (in Bauru municipality) (Supplementary Material, Table S1), each one containing: one fragment of native *cerradão*; one area of livestock pasture planted with the African grass species *Urochloa decumbens*, known as brachiaria; and one area of *Eucalyptus* plantation (*Eucalyptus urophylla* or *E. grandis* or *E. urograndis* or *E. saligna*) (Figure 2).

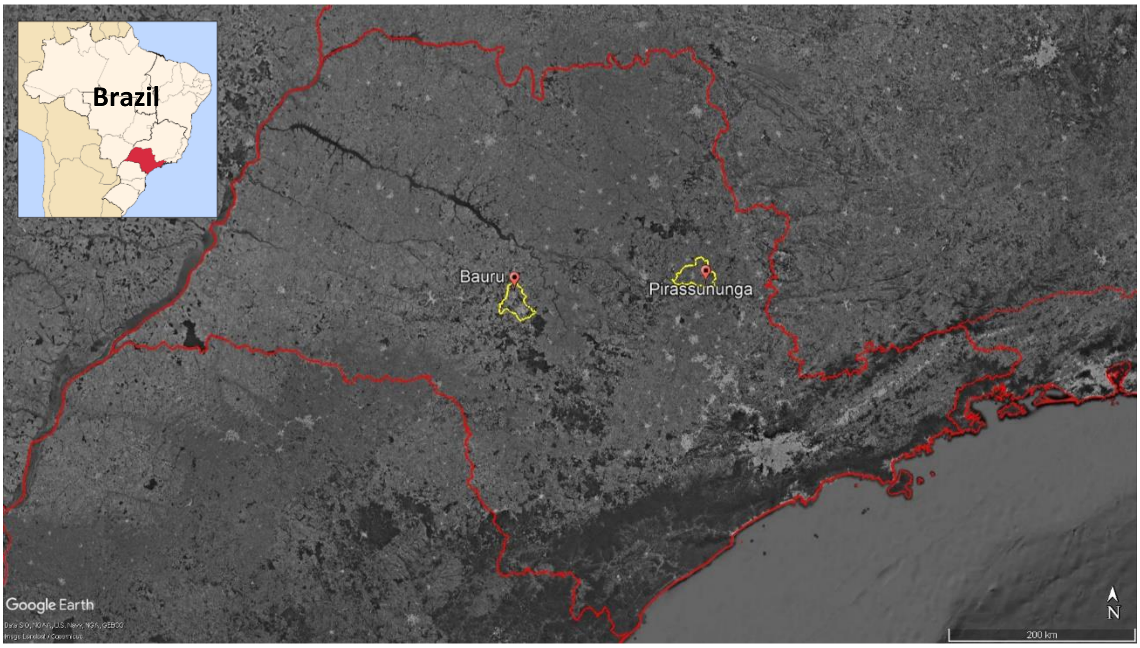

Figure 1. Location of the study area, municipalities of Pirassununga and Bauru, São Paulo state, Brazil (Google Earth, 2018).

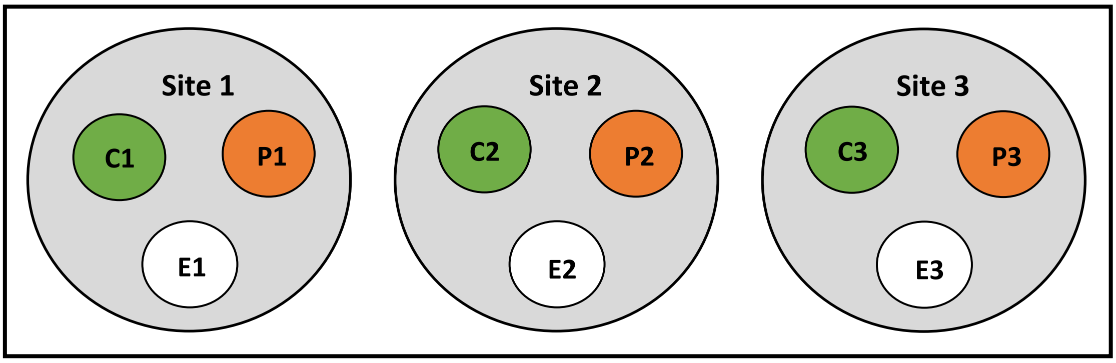

Figure 2. Schematic drawing of the three selected sites containing one fragment of native *cerradão* (C); one area of livestock pasture (P); and one area of *Eucalyptus* plantation (E).

All sites were located on flat to gently undulating terrains (slope between 0% and 5%), and on dystrophic Red and Dark-Red Latosols (according to Oliveira *et al*. 1999), with sand content over 80% (dominated by coarse sand) and pH ranging from 4.95 to 5.93 (Supplementary Material, Table S2). The average bulk density from 0-5 and 30-35 cm depth for Cerrado was 1.21 $g.cm^{-3}$, while the target pastures had 1.30 $g.cm^{-3}$ and *Eucalyptus* plantations had 1.27 $g.cm^{-3}$. Soil bulk density values for the two sampled depths (0-5 and 30-35 cm) and study sites are available in Table S2 (Supplementary Material). Regarding the fertilization procedures, pastures received N-P-K only at establishment, or did not receive any fertilization, while the *Eucalyptus* plantations received liming and N-P-K at establishment and, according to the site, none, one or two extra inputs after planting (Table S1). Mean concentrations of soil P, N and organic matter (SOM) for the target Cerrado areas, pastures and *Eucalyptus* plantations, up to 200 cm depth, can be found in Table S3 (Supplementary Material). Soil P and N showed a decrease trend according to depth, with higher values in the first 10 cm, where we can find concentrations ranging from 5.73 $mg.kg^{-1}$ of P and 0.78 $g.kg^{-1}$ of N for Cerrado, 7.10 $mg.kg^{-1}$ and 0.62 $g.kg^{-1}$ for pastures, and 9.05 $mg.kg^{-1}$ and 0.70 $g.kg^{-1}$ for *Eucalyptus* plantations. SOM concentrations also decreased with depth, with higher values at surface (0-10 cm), being 1.11%, 1.08% and 1.05% respectively for Cerrado, pastures and *Eucalyptus*.

Experimental design and soil sampling

We used a randomized block design, with three fixed factors: LULC (Cerrado, Pasture, and *Eucalyptus*), soil depth (0-10, 10-30, 30-50, 50-100, and 100-200 cm) and season (dry and wet), and one random factor: site. For each LULC and site, we established a 140 m transect, along which we placed regularly spaced sampling points (Figure 3), being four sampling points in the dry season (August to October of 2015) and three in the wet season (April to May of 2016). Using an auger of 17 cm in diameter, we sampled soils at 0-10 and 10-30 cm depth from all sampling points (four in the dry season and three in the wet season), plus at 30-50, 50-100 and 100-200 cm depths from two sampling points for each season. Soil samples were sieved at 2 mm, and separated in two sub-samples: one moist-soil sample kept at 4º C in closed plastic bag until analyses, and one dry-soil sample that was air-dried and stored at room temperature prior to analyses. Extra undisturbed soil samples were collected at 0-5 and 30-35 cm depth (three samples per depth, LULC and site) and dried at 105°C (volumetric ring method; Blake and Hartge 1986) to calculate soil bulk density for the uppermost soil layers (0-10, 10-20, and 30-50 cm).

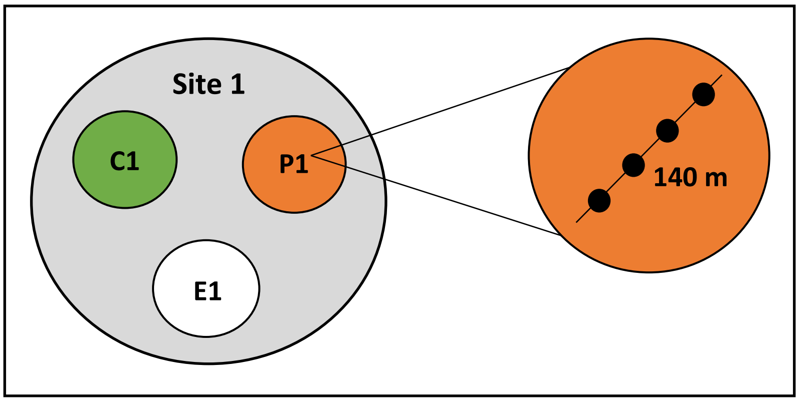

Figure 3. Schematic drawing of the LULC in each site where we established a 140 m transect with regularly spaced sampling points, being four sampling points in the dry season (August to October of 2015). C=*cerradão*; P=pasture; E=*Eucalyptus*.

Soil analyses

The microbial biomass carbon (MBC) was quantified through the method proposed by Witt *et al*. (2000), that includes extraction by fumigation at atmospheric pressure (fumigation without vacuum application) and colorimetric determination by potassium dichromate oxidation. The biomass values obtained were converted into the amount of C stored by the following equation:

$C\text{-mic} = (C_f - C_{nf})/k_c$

where C_f = carbon of the fumigated sub-sample, C_{nf} = carbon of the non-fumigated sub-sample, and kc = correction factor, 0.33, expressed as mgC.soil kg^{-1}.

The non- fumigated sub-sample from the MBC analyses was considered as dissolved organic carbon (DOC). SOM content was determined by the Walkley-Black method (Walkley-Black 1934) and SOC was calculated from SOM results, using the correction factor = 1.74 (Nelson and Sommers 1982). The microbial quotient (MBC:SOC), that reflects the microbial biomass contribution to soil organic carbon, was calculated by dividing MBC values by SOC results (Anderson and Domsch 1990). We also calculated the ratio DOC/SOC, which indicates SOC lability.

Soil basal respiration (SBR) was estimated by the Emteryd (1989) method, based on the absorption of CO_2 by NaOH solution, in fresh soil samples, at the laboratory. Soil samples (5g) were incubated for 96 h in sealed flasks containing 3 ml of 0.1 M NaOH aqueous solution. After incubation, 2 ml of $BaCl_2$ were added to the NaOH solution, to stabilize the CO_2 retained in the form of barium carbonate. The alkaline solution coming from the reaction between CO_2 and $BaCl_2$ was then titrated with standard 0.1 N HCl, using phenolphthalein as indicator.

We then calculated the metabolic quotient (qCO_2), which is the ratio between SBR and MBC, and measures the respiration rate per unit of microbial biomass, representing the energy necessary to maintain metabolic activity in relation to the energy used for synthesizing biomass. Therefore, it is used as an indicator of microbial efficiency (Bardgett and Saggar, 1994).

*A*dditionally, other soil parameters were determined, such as soil texture, by the Bouyoucos densimeter method at 5-10 cm depth (Camargo *et al*., 1986), soil total nitrogen content, by the microkjeldahl method, and the available phosphorus (P), by atomic absorption spectrophotometry (following Raij *et al*., 1987).

Data Analyses

Data from the different sampling points were averaged for each site and depth, so that only a single value per depth, LULC and site remained. For the variables of interest (DOC, MBC, SBR, qCO_2, MBC:SOC and DOC/SOC) we analyzed the main effects and interactions of LULC (Cerrado, Pasture or *Eucalyptus* plantation), soil depth (five depths) and season (dry and rainy), using a mixed-factor analysis of variance, with LULC, depth and season as fixed factors, and site as random factor. A Principal Components Analysis (PCA) was performed on DOC, MBC and SBR values from soils top 30 cm by plotting each sampling point against the two main axes of the PCA, with the objective of understanding how LULC, between- and within-site spatial distinctions, and seasonality contributed to soil pools and activity. The relationships between soil C variables (DOC, MBC and SBR) were then analysed by Pearson’s correlations. We used the SPSS 20.0 (for Windows) for statistical analyses.

Results

SOC was affected by depth ($F=3.5$; $p=0.063$) and the values were slightly higher in Cerrado (Table 1), however, not significantly different among the three LULC ($F=0.849$; $p=0.493$). Considering both seasons together, LULC effect was significant for the microbial biomass carbon (MBC) and for the microbial quotient (MBC:SOC) (Table 2), and that effect was stronger in the dry season (Table 3); for both variables Cerrado had higher values than pastures and *Eucalyptus* plantations, which showed similar results

(Figure 4). In fact, all C variables except soil basal respiration (SBR) were subjected to significant seasonal effect (Table 2). Dissolved organic carbon (DOC), DOC:SOC ratio, SBR and the metabolic quotient (qCO_2) showed higher values in the dry season, while MBC and MBC:SOC had higher values in the wet season. The interaction between season and site showed significant effect on SBR (Table 2), and that significant effect is observed in the dry season (Table 3). Also, there was significant interaction between land use, season and site (Table 2). Depth effect was only marginally significant for the qCO_2 (Table 2), with clearer pattern in the dry season (Figure 4, Table 3), for which the three land uses showed lower values up to 50 cm (Figure 5). The qCO_2 was lower for Cerrado and higher for *Eucalyptus* plantations in almost all depths (Figure 5).

No significant effect of depth on other C variables resulted when analyzing both seasons together, which was not expected. However, analyzing the seasons separately, depth effect is observed in DOC (wet season) and MBC (dry season) (Table 3). For DOC, the interaction between depth and LULC resulted highly significant in the wet season, as well as with site, indicating distinct depth profiles according to land uses or sites. Cerrado showed a sharp decrease of DOC from 10 to 50 cm (not changing much below 50 cm), while Pasture and *Eucalyptus* showed an increase from 10 to 30 cm, and below 50 cm DOC values were very similar for all land uses (Figure 4). The interaction between depth and LULC was also marginally significant for the ratio DOC:SOC in the wet season (Table 3), showing patterns very similar to those of DOC (Figure 4). Still in the wet season, significant effects of the interaction between depth and site were detected on DOC, DOC:SOC and MBC:SOC, indicating differences among depth profile within sites (Table 3).

According to different land uses, Cerrado shows in both seasons the expected decreasing pattern with depth for most C variables, but Pasture and *Eucalyptus* do not, as they presented lower figures at soil surface (Figure 4), and that is probably why depth effect was not significant when considering all land uses in the overall analysis. The interaction between soil depth and season was marginally significant for the qCO_2 in the overall analysis (Table 3) due to patterns in the dry season (Table 3, Figure 4), reflecting different soil profiles among seasons. The MBC:SOC had a decreasing pattern with soil depth at the dry season (Figure 4) but this effect was not significant (Table 3). Significant interactions involving season and site also occurred (Table 3).

Table 1. Soil organic carbon (SOC) mean values for three sites sampled only at the dry season; five depths and three LULC (Cerrado, Pasture and Eucalyptus). (n=3) (Means ± Standard error).

LULC	Depth (cm)	SOC ($g.kg^{-1}$)
Cerrado	10	6.405 ± 0.884
	30	5.446 ± 0.395
	50	5.172 ± 0.283
	100	3.943 ± 0.146
	200	4.393 ± 1.147
Pasture	10	6.218 ± 0.279
	30	5.314 ± 0.346
	50	4.682 ± 0.356
	100	3.953 ± 0.760
	200	2.716 ± 0.744
Eucalyptus	10	6.028 ± 0.914
	30	4.938 ± 0.872
	50	4.733 ± 0.307
	100	4.843 ± 0.627
	200	2.650 ± 0.926

Table 2. Results of mixed-effects Analysis of Variance on C pools and microbial activity indicators; F statistic and p value for main effects and interactions. In bold: significant effects; in bold and italics: marginally significant ($p \leq 0.1$) effects. DOC= Dissolved organic carbon; MBC=Microbial organic carbon; SBR=Soil basal respiration; SOC= Soil organic carbon; qCO2= Metabolic quotient.

Effect	**DOC**	**MBC**	**MBC:SOC**	**DOC:SOC**	**SBR**	**qCO_2**
LULC	F=0.5 p=0.628	F=16.1 **p= 0.012**	F=6.7 **p=0.053**	F=0.9 p=0.459	F=0.7 p=0.544	F=1.4 p=0.336
Depth (D)	F=2.3 p=0.149	F=2.4 p=0.137	F=0.6 p=0.683	F=1.3 p=0.351	F=2.3 p=0.141	F=3.1 ***p=0.080***
Season (Se)	F=393.6 **p=0.003**	F=59.9 **p=0.016**	F=305.5 **p=0.003**	F=111.2 **p=0.009**	F=6.4 p=0.128	F=26.4 **p=0.036**
Site (St)	F=1.4 p=0.473	F=1.3 p=0.395	F=1.5 p=0.481	F=0.8 p=0.532	F=2.0 p=0.360	F=1.5 p=0.591
LULC x D	F=0.8 p=0.590	F=1.6 p=0.196	F=0.7 p=0.717	F=1.3 p=0.299	F=1.5 p=0.234	F=0.6 p=0.761
LULC x Se	F=0.1 p=0.891	F=0.8 p=0.509	F=0.4 p=0.714	F=0.2 p=0.830	F=0.02 p=0.978	F=1.4 p=0.344
LULC x St	F=0.8 p=0.567	F=O.4 p=0.798	F=0.7 p=0.634	F=0.5 p=0.723	F=0.5 p=0.734	F=0.8 p=0.595
D x Se	F=0.3 p=0.893	F=2.4 p=0.132	F=2.8 p=0.103	F=0.9 p=0.482	F=1.6 p=0.264	F=2.8 ***p=0.098***
D x St	F=0.6 p=0.722	F=0.4 p=0.909	F=1.8 p=0.197	F=0.4 p=0.857	F=0.9 p=0.562	F=0.8 p=0.644
Se x St	F=0.1 p=0.876	F=0.3 p=0.716	F=0.8 p=0.930	F=0.4 p=0.679	F=7.1 **p=0.050**	F=2.1 p=0.494
LULC x D x Se	F=0.8 p=0.623	F=0.5 p=0.845	F=0.7 p=0.691	F=1.9 p=0.124	F=0.8 p=0.643	F=0.6 p=0.756
LULC x D x St	F=0.9 p=0.603	F=1.1 p=0.402	F=1.7 p=0.152	F=1.2 p=0.372	F=1.0 p=0.497	F=1.0 p=0.489
LULC x Se x St	F=3.1 **p=0.045**	F=2.2 p=0.115	F=1.5 p=0.253	F=2.6 ***p=0.075***	F=7.5 **p=0.001**	F=0.9 p=0.462
D x Se x St	F=1.3 p=0.331	F=1.9 p=0.115	F=2.4 **p=0.068**	F=2.0 p=0.111	F=1.0 p=0.499	F=0.5 p=0.807

Table 3. Results of mixed-effects Analysis of Variance on C pools and dynamic indicators for the dry and wet seasons separately; F statistic and p value for main effects and interactions. In bold: significant effects; in bold and italics: marginally significant (p≤0.1) effects. DOC= Dissolved organic carbon; MBC=Microbial organic carbon; SBR=Soil basal respiration; SOC= Soil organic carbon; qCO2= Metabolic quotient; LULC=Cerrado, Pasture or Eucalyptus; D=Depth; St=Site.

		LULC	**D**	**St**	**LULC x D**	**LULC x St**	**D x St**
DOC	**DRY**	F=0.3 p=0.785	F=0.4 p=0.796	F=0.2 p=0.851	F=0.6 p=0.790	F=3.0 **p=0.048**	F=0.9 p=0.567
	WET	F=1.4 p=0.351	F=3.0 ***p=0.085***	F=0.2 p=0.826	F=5.6 **p=0.002**	F=2.5 ***p=0.082***	F=5.1 **p=0.003**
MBC	**DRY**	F=24.7 **p=0.006**	F=9.8 **p=0.004**	F=14.0 p=0.590	F=1.6 p=0.211	F=0.6 p=0.678	F=0.6 p=0.748
	WET	F=2.1 p=0.238	F=0.8 p=0.542	F=0.3 p=0.779	F=0.8 p=0.605	F=2.0 p=0.142	F=1.7 p=0.175
MBC:SOC	**DRY**	F=6.6 ***p=0.054***	F=1.2 p=0.361	F=2.8 p=0.264	F=0.4 p=0.872	F=1.1 p=0.384	F=0.8 p=0.590
	WET	F=1.9 p=0.255	F=1.2 p=0.364	F=0.2 p=0.799	F=0.8 p=0.625	F=1.1 p=0.396	F=3.9 **p=0.010**
DOC:SOC	**DRY**	F=0.8 p=0.522	F=1.8 p=0.230	F=0.2 p=0.795	F=1.5 p=0.222	F=1.1 p=0.369	F=1.8 p=0.146
	WET	F=0.05 p=0.950	F=0.3 p=0.890	F=2.02 p=0.211	F=2.7 **p=0.045**	F=1.0 p=0.425	F=2.8 **p=0.037**
SBR	**DRY**	F=0.09 p=0.912	F=2.2 p=0.158	F=14.5 **p=0.014**	F=1.4 p=0.263	F=7.4 **p=0.001**	F=1.07 p=0.429
	WET	F=1.4 p=0.335	F=1.1 p=0.407	F=3.2 p=0.200	F=0.5 p=0.827	F=2.1 p=0.128	F=0.6 p=0.725
qCO_2	**DRY**	F=1.4 p=0.341	F=3.0 ***p=0.089***	F=3.0 p=0.532	F=0.6 p=0.759	F=0.9 p=0.506	F=0.5 p=0.835
	WET	F=3.0 p=0.157	F=0.9 p=0.521	F=1.4 p=0.401	F=0.9 p=0.542	F=0.9 p=0.484	F=1.0 p=0.463

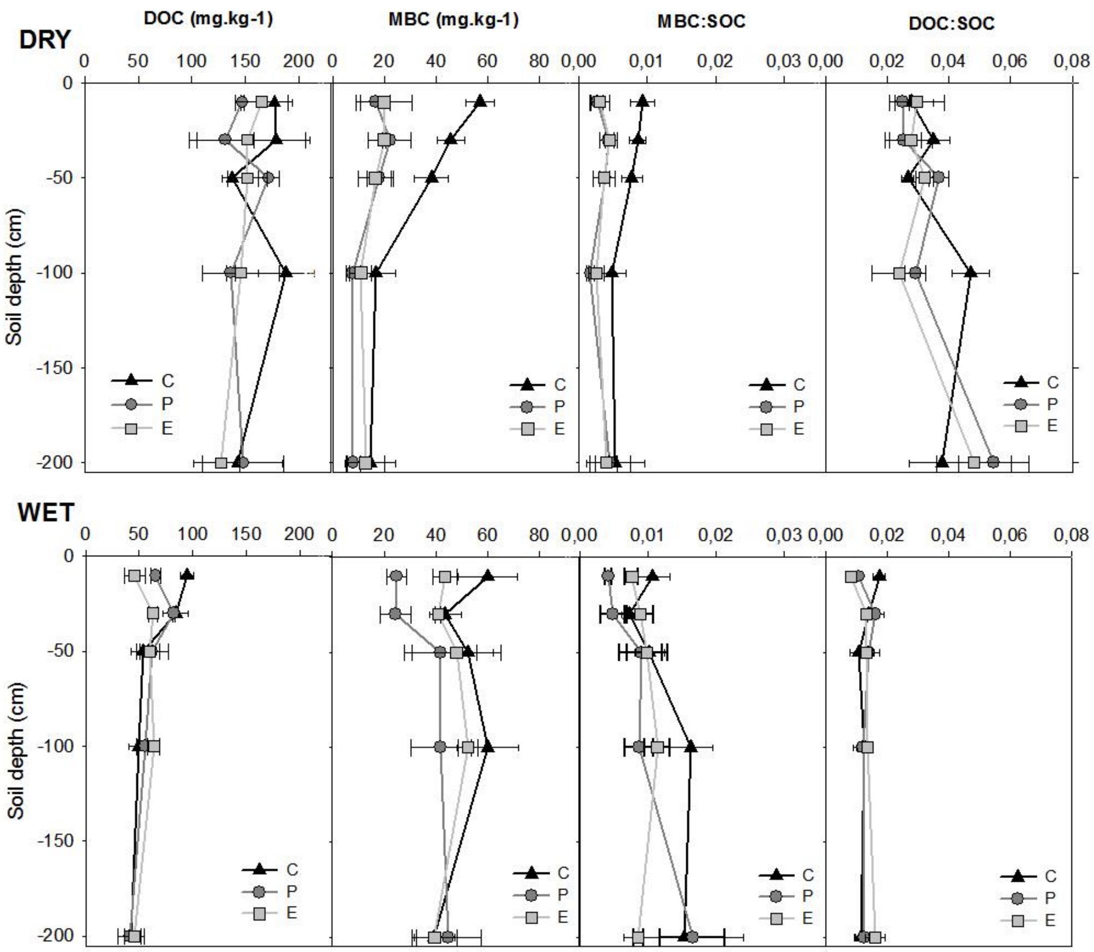

Figure 4. Patterns of soil DOC and MBC pools, MBC:SOC and DOC:SOC ratio, according to depth for the target LULC (Cerrado, Pasture and Eucalyptus) in the dry and wet seasons. Values are means ± standard error (n = 3). DOC= Dissolved organic carbon; MBC= Microbial biomass carbon; SOC= Soil organic carbon; C= Cerrado; P= pasture; E= Eucalyptus.

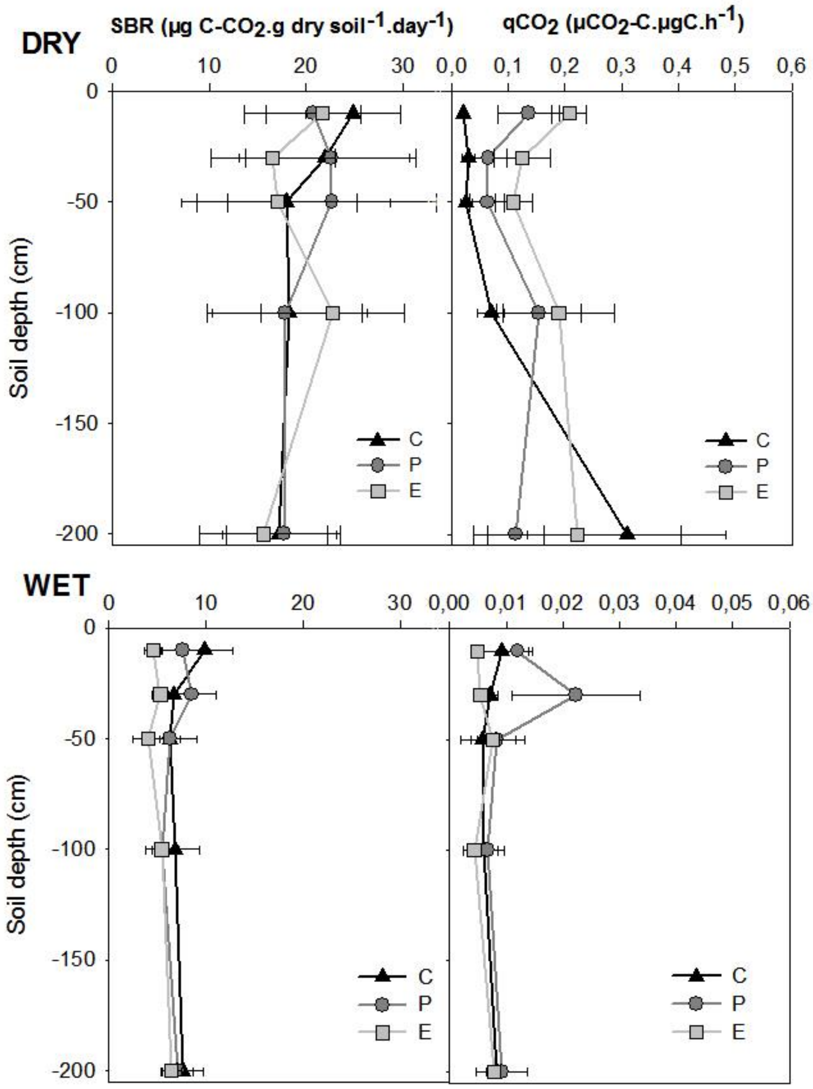

Figure 5. Patterns of soil SBR and qCO2 according to depth for the three target LULC (Cerrado, Pasture and Eucalyptus) in the dry and wet seasons. Values are means ± standard error (n=3). SBR= Soil basal respiration; qCO2= Metabolic quotient; C= Cerrado; P= Pasture; E= *Eucalyptus*.

Considering the first 30 cm of the soil profile, and both seasons together, the conversion of Cerrado to pasture and *Eucalyptus* plantations resulted in an overall reduction in the stocks of the labile fraction of SOC (DOC and MBC) (Table 4). Pastures had 54% decrease in the MBC stocks compared to Cerrado, while Eucalyptus plantations had 36% reduction (Table 4). Both land uses had similar reductions in the DOC stocks, pastures had 15% decrease and *Eucalyptus* plantations 13% (Table 4).

Table 4. Average stocks of labile SOC (DOC and MBC) in the 0-30 cm layer for Cerrado, Pasture and *Eucalyptus*, and the percentages losses (between parentheses, negative sign) for the respective carbon variable for Pasture and Eucalyptus relative to Cerrado.

LULC	DOC ($Mg.ha^{-1}$)	MBC ($Mg.ha^{-1}$)
Cerrado	0.49 ± 0.04	0,18 ± 0.02
Pasture	0.41 ± 0.06 (-15%)	0,08 ± 0.01 (-54%)
Eucalyptus	0.42 ± 0.05 (-13%)	0,11 ± 0.01 (-36%)

Exploring the effect of the interactions among land use, depth and site separately for the dry and wet seasons we see that in both seasons the interaction between LULC and site had a marginally significant effect on DOC (Table 2), indicating different land use effects within sites. DOC values were usually higher for Cerrado, and *Eucalyptus* show higher values than Pasture in the dry season but the opposite in the wet season at higher depths (Figure 4). SBR also had a significant effect of the interaction between land use and site in the dry season (Table 2), with higher values associated to Cerrado at the upper layers, and similar values to the other land uses at higher depths. Contrarily, Pasture and *Eucalyptus* showed the lowest values associated to the top soils (Figure 5).

A marginal Correlation between SBR and MBC was detected in the dry season (p= 0.064) when analyzing seasons separatly (Figure 6), however, when cosidering both seasons togheter, C variables are positively correlated to each other: MBC and DOC (R^2= 0.251; F= 30.8; p<0.001), SBR and DOC (R^2= 0.359; F=50.9; p<0.001), SBR and MBC (R^2= 0.059; F=6.6; p=0.012).

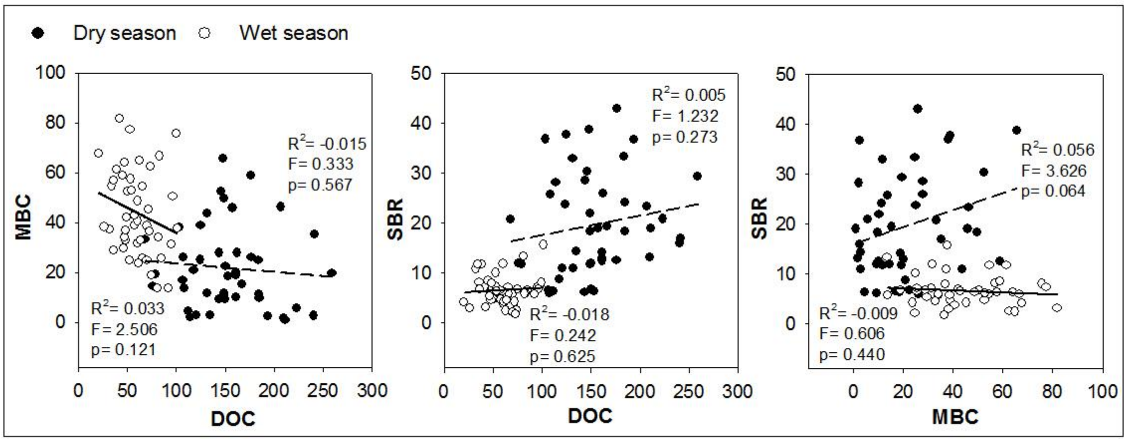

Figure 6. Pair-wise Pearson's correlation coefficients (and R2, F and p values) for dissolved organic carbon (DOC), microbial biomass carbon (MBC) and soil basal respiration (SBR) in the dry and wet seasons. Data are averages per depth, LULC and site (n=45 for each season).

The PCA ordination of the top 30 cm sampling points reflects the contribution of LULC and season to the variation of C variables (Figure 7). Seasonal variation is indicated by the first axis (PC1), which was positively correlated to DOC and MBC, showing higher values associated to the dry season. The variation due to LULC was captured by the second axis (PC2), which was positively correlated to MBC, showing a tendency to higher values for Cerrado areas, in both seasons. In the wet season, Pasture values were the lowest, while in the dry season pasture and *Eucalyptus* showed similar results.

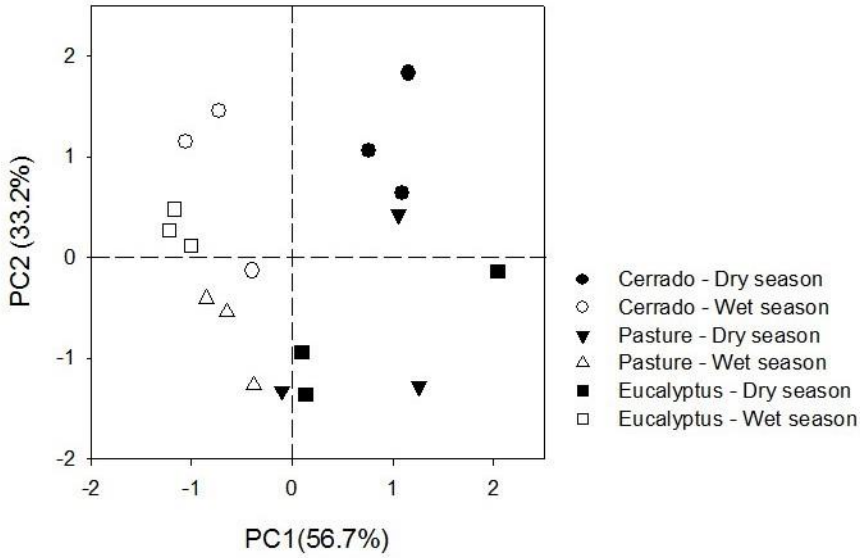

Figure 7. PCA-based ordination of sampling plots (symbols) for each LULC (Cerrado, Pasture and *Eucalyptus*) and season (dry, wet) based on linear combinations of dissolved organic carbon (DOC), microbial biomass carbon (MBC) and soil basal respiration (SBR), for the 0-30 cm depth.

Discussion

The conversion of native Cerrado vegetation (*cerradão* physiognomy) into pastures and *Eucalyptus* plantations reduced the dissolved organic carbon (DOC) pool, the microbial biomass carbon (MBC) and the microbial quotient (MBC:SOC), and more pronouncedly in the upper soil layers.

The decline on the upper soil DOC pool due to Cerrado conversion to agricultural uses was highly influenced by spatial and temporal variations. Contrarily to our results, Vinhal-Freitas *et al*. (2017) found no significant differences on DOC values when comparing native Cerrado vegetation and *Urochloa brizantha* pasture in the wet season. On the other hand, Dieckow *et al*. (2005) and Souza *et al*. (2009) reported rapid reductions in labile carbon stocks associated to Cerrado land convertion, followed by rapid recovery over time, indicating soil C dynamics. Silva *et al*. (2011) also resported LULC effect on the labile fraction of SOC for the top 10 cm depth when analysing integrated crop-livestock areas in the Cerrado biome. DOC comes from the dissolved organic matter such as plant residues, root exudates, microbial degradation products and decomposing litter, therefore, higher values are expected at surface (Jobbágy and Jackson, 2000; Chantigny, 2003). DOC plays an important part in the global C cycle (Siemens 2003); due to its lability and solubility it is an essential source of carbon to microbes (Chantigny, 2003; Kalbitz *et al*., 2000; Straathof *et al*., 2014) and an indicator of the dynamics of SOC pools (Strosser, 2010; Schiedung *et al*., 2017). Analyzing 190 arable soils from Hungary, Filep *et al*. (2015) demonstrated the high potential of DOC on indicating soil quality and fertility. The higher values of DOC on Cerrado soils, especially at surface, are then associated to higher quantities of a more diverse litter and root exudates, and denote higher soil quality. Lower values of DOC:SOC were also found in pastures and *Eucalyptus* plantations compared to Cerrado, that can also reflect alterations in litter quantity and quality, soil compaction and soil microclimate.

Pastures and *Eucalyptus* plantations also showed much lower figures related to MBC, compared to Cerrado. These findings are compatible with the just mentioned DOC results, and are in agreement with other authors who reported MBC reductions for native Cerrado converted to low productivity brachiaria pastures and *Eucalyptus* plantations in Brazil (Alvarenga *et al*., 1999; Muniz *et al*., 2011; Oliveira *et al*., 2016; Ribeiro *et al*., 2010; Vinhal-Freitas *et al*., 2017). Under natural conditions, Cerrado soils offer a better environment for microbial growth, such as high floristic diversity and a better quality litter, less soil disturbance (no agricultural management) that favors litter accumulation and root development, and the maintenance of soil temperature and moisture due to the presence of trees and accumulated litter. On the other hand, MBC reduction in pastures and *Eucalyptus* plantations – that was more pronounced in the dry season – indicates lower substrate availability and/or unfavorable conditions for microbial growth. Values of MBC were mostly higher in the wet season for the three LULC, probably related to more favorable conditions to microbial activity and biomass development, such as soil moisture, higher soil temperature and more substract available (Cattelan and Vidor, 1990; Mazzarino *et al*., 1991). Higher MBC values for Cerrado vegetation in the wet season have been reported previously (Nardoto and Bustamante, 2003; Gama-Rodrigues *et al*., 2005; Carvalho, 2005), and also for Cerrado converted to *Urochloa* pastures (Frazão *et* *al*., 2010) and *Eucalyptus* plantations (Gama-Rodrigues *et al*., 2005). So, our data indicate that Cerrado convertion to pasture and *Eucalyptus* plantation influenced the MBC concentration more strongly under dry conditions, a similar result found by Frazão *et al*. (2010) when comparing Cerrado vegetation to a low productivity *Urochloa brizantha* pasture that had been used for 22 years. Since soil microorganisms play a fundamental role in the organic matter transformation, and nutrients release and immobilization (Jenkinson and Ladd, 1981), processes such as decomposition, pollutants degradation, nutrient cycling, and energy flow within the soil may be impaired under agricultural uses.

Similarly to MBC stocks, the microbial quotient (MBC:SOC) decreased with the conversion of Cerrado vegetation into pastures and *Eucalyptus* plantations, indicating that land use change reduced the contribution of microbial biomass to total soil organic carbon (Anderson and Domsch, 1989), which would modify SOC turnover, since microbial community is the most active component in soil biochemical process (Gougoulias *et al*., 2014). Higher value of MBC:SOC in Cerrado suggests greater C conversion to MBC and indicates organic C stability in these long-time undisturbed soils (Kalambukattu *et al*., 2013). Pastures and *Eucalyptus* plantations had similar MBC:SOC values in the dry season, in both cases lower than those of the Cerrado all over the soil depth profile; this declines on MBC:SOC can be related to SOM reductions (Brookes, 1995) and/or changes in the SOM quality, which hampers the use of SOC by the microbial community (Gama-Rodrigues *et al*., 2008). Whereas, in the wet season, higher MBC:SOC values were found in *Eucalyptus* plantations compared to pastures, indicating higher efficiency of *Eucalyptus* plantations than pastures in converting SOC to microbial biomass.

According to Jenkinson and Ladd (1981), MBC usually comprises 1 to 5% of the total SOC stock, however, in our study MBC:SOC was ≥1% only in the Cerrado vegetation, during the wet season. Alvarenga *et al*. (1999) recorded MBC:SOC values around 2 to 3% of SOC for Cerrado, Pasture and *Eucalyptus* plantations.

MBC, MBC:SOC, DOC and DOC:SOC values were highly influenced by seasonality, and also their patterns along the soil profile, that might be attributed to differences in vegetation conditions, and specially to soil water status, soil temperature, which are determinant factors for microbial activities.Soil DOC values were generally higher in the dry season; despite not showing significant response to season values of SBR were higher at dry , what could suggest that soil water content was not a limiting factor under drier conditions. Both the mixed-factor analysis of variance and the PCA revealed a clear effect of seasons for every land use. DOC values were higher in the dry season for all land uses. In the dry season, plants lose their leaves as a mechanism to save water (Reich and Borchert, 1984), which results in more leaf litter accumulated on soil (Apelbaum and Yang 1981). Greater amount of litter would lead to increases soil organic matter and maintains soil moisture, and subsequent raise of DOC concentrations. According to Li *et al*. (2018), DOC is the most effective dissolved C compartment to characterize seasonality in the organic matter. Contrarily, MBC results were higher in the wet season, when conditions for microbial growth – humidity and higher temperatures – are more favorable.

The effect of depth on MBC was significant in the dry season and marginally significant for DOC in the wet season, however, the patterns of all these variables along soil depth clearly differed according to season (evident in Figure 1). The expected pattern of these C-variables is a decreasing gradient with depth, as consequence of litter accumulation and fine roots exudates, a pattern that was clearer for Cerrado. Other studies have reported higher concentrations of MBC in the top layers for Cerrado, with sharp decreases according to depth related to organic matter availability (Resck and Silva, 1995; Resck, 1997; Corazza *et al*., 1999; Ferreira *et al*., 2007); Li *et al*. (2018) found a common pattern of decreased DOC concentrations along soil depth with considerably lower levels from 30 cm downwards in a revision of 120 articles from all over the world. However, in the pastures and *Eucalyptus* plantations higher values of DOC, MBC, MBC:SOC and DOC:SOC often did not occur in the first soil layer (0-10 cm) but in the subsequent ones (10-30 or 30-50 cm). This kind of "reversed" profile could be a consequence of the agricultural use of those areas, causing impoverished conditions in the soil surface. Changes in litter quality and quantity are expected in consequence of changing the species composition, especially when transforming a natural biodiverse environment on an exotic species monoculture; mechanization (in *Eucalyptus* plantations) and trampling (in pastures) lead to soil compaction ; fertilization may have caused nutrient accumulation in the sub-superficial layers. Higher values of nitrogen and soil bulk density for pastures and especially for *Eucalyptus* plantations were registered, reinforcing this perception.

The replacement of native Cerrado vegetation by *Urochloa* pastures and *Eucalyptus* plantations significantly affected soil basal respiration (SBR) under the influence of spatial variables (site) and seasonal conditions, as pointed by the mixed-factor analysis of variance and PCA. Plant and microbial metabolism interact and are related to weather variation, mostly temperature and water content (Raich and Potter, 1995; Davidson *et al*., 2000; Medvigy *et al*., 2010), which makes it highly sensitive to uncontrolable environment variations that resulted on the observed seasonal effect on soil respiration. In our case we chose a method for mesuring the SBR that controlled at least the temperature, as soil incubation period occurred in the laboratory. According to Wei *et al* (2015) and Saiz *et al*. (2006) soil temperature is the predominant factor to drive seasonal variations on soil respiration. In the present study we soil respiration was determined under field moisture conditions, , therefore, the low SBR values we observed in the wet season may be explained by the high soil water content in that season, that may have limited soil respiration by low oxygen availability for microbial activities (Saiz *et al*., 2006). On the other hand, we found greater SBR values in the dry season for all land uses.

According to Islan and Weil (2000), high respiration rates can be associated to both greater levels of productivity in the system and ecological unbalance. Our results show, in the dry season, positive correlation between SBR and MBC, indicating higher microbial activity associated to microbial biomass generation, which was more evident in the Cerrado. Other authors also noted the seasonal effect on SBR according to different LULC, however, unlike us, higher SBR variations were found in the wet season. For example, comparing Cerrado and *Eucalyptus* plantations, Cortez (2013) reported higher SBR values for *Eucalyptus* plantations in the wet season; Frazão *et al*. (2010) found higher SBR values in Cerrado during the wet season when comparing it with degraded pastures. Like us, Vinhal-Freitas *et al*. (2017) found no significant differences of SBR values between Cerrado and *Urochloa brizantha* pasture in the wet season.

When considering both seasons together, all C variables (DOC, MBC and SBR) were positively correlated to each other, indicating seasonality. In this case, we can assume that in the dry season higher DOC stocks and higher SBR rates indicate there is higher microbial activity decomposing the available C (sometimes more effectively, depending on the land use and associated spatio-temporal variations) with consequent MBC production by the end of the wet season, when DOC and SBR values decline. According to Ren *et al*. (2018), SBR changes are intimately connected to DOC concentrations, being mainly controlled by the microbial community.

As happened with the SOC labile pools (DOC, MBC, MBC:SOC and DOC:SOC), a very evident effect of the Cerrado conversion to agricultural uses was the reversal of the expected pattern for SBR (decreasing values according to soil depth) along the soil profile: Cerrado showed higher DOC, MBC and SBR at surface (10 cm), while pastures and *Eucalyptus* plantations had higher concentrations at 30 cm depth. In fact, higher qCO_2

values were found in pastures and *Eucalyptus* plantations in the first soil layer. As higher qCO_2 could be associated to soils under disturbance or stress (as the microbial community spends more C for maintenance than for synthesizing biomass) those high values of qCO_2 can be linked to lower substrate quantity or quality, and/or to unfavorable environmental conditions, such as soil compaction or adverse soil microclimate.

The qCO_2 was also affected by season, with higher rates in the dry season for Cerrado, pastures and *Eucalyptus* plantations. Frazão *et al*. (2010) also reported higher qCO_2 for *Urochloa* pastures and native Cerrado in the dry season. Higher concentrations of easily degradable C are known to cause qCO_2 increase (Ocio and Brookes, 1990; Thirukkumaran and Parkinson, 1999). Considering our results for DOC, that were higher in the dry season for all land uses, qCO_2 rates could be associated to greater amounts of available labile C.

Depth also affected the qCO_2. In the more superficial soil layers the microbial efficiency barely changed for all LULC. However, qCO_2 values increased from 50 cm belowground, especially in the dry season and for Cerrado. In the wet season, qCO_2 variations with depth were low except for pastures, whose values were much higher at 30 cm depth and associated to a particular site, reflecting the strong spatio-temporal variation in soil functioning. Some studies have reported that pastures converted from native Cerrado under a proper management can stock high quantities of C, even higher than the native vegetation itself (Roscoe *et al*., 2001; Neufeldt *et al*., 2002). However, without appropriate management, pastures especially on coarse textural soils get into rapid degradation process (Silva *et al*., 1994; Dieckow *et al*., 2009) and sustain lower C stocking rates (Salton, 2005), that can lead to nutrient decline and C emissions to the atmosphere. The pasture sites analyzed in this research had been fertilized only once at least 15 years before sampling. Despite brachiaria high adaptability to low nutrient soils, there is a need of fertilization or alternative cultivation practice (e.g. rotation system) to maintain its productivity. Thus, our results draw attention to possible negative effects on soil organic matter decomposition, nutrient cycling and C stocks on soils under improper management.

Conclusion

The conversion of Cerrado ecosystems into brachiaria pastures and *Eucalyptus* plantations showed great alterations on the amounts and patterns of soil organic C- labile fractions (MBC, MBC:SOC, DOC, DOC:SOC and qCO_2) along the soil profile up to 30 and 50 cm depth. Soil compaction and deteriorated conditions for microbial growth perhaps resulted from cattle trampling in pastures and machine traffic in *Eucalyptus* plantations. Reduced values of MBC and MBC:SOC indicate lower C conversion to microbial biomass and less SOC stability in both land uses. Higher SBR and qCO_2 rates in the target land uses are related to a microbial community under stress.

DOC changed with land use in different ways among sites, demonstrating high sensibility to spatio-temporal variations, with reductions for pastures and *Eucalyptus* plantations recorded especially in the top 10 cm.

Depth effect was only significant for MBC and DOC, and Cerrado showed a clear decreasing gradient with depth while the other land uses showed a “reversed” pattern in the 30-50 cm upper layers. Although most variables stabilized below 100 cm depth, high values of qCO_2 in Cerrado from 100 cm revealed signs of stressful conditions for microbial growth in deeper soils. Our results highlight the importance of considering deeper layers when assessing microbial activity and carbon stocks in the soil.

Seasonality influenced soil functioning and affected all C variables assessed in this research; land use effect on SOC labile pools was more evident in the dry season, when higher DOC concentrations and SBR rates were recorded. On the other hand, higher MBC stocks occurred in the wet season. We assumed that the DOC produced in the dry season was consumed by the microbial biomass, producing higher SBR rates (higher microbial activity), favoring MBC development in the wet season, and DOC and SBR reductions. These dynamic was more efficient in Cerrado, which had higher MBC and MBC:SOC rates, and lower qCO_2 values. The qCO_2 was also higher in the dry season indicating higher microbial activity (associated to DOC stocks) and/or a microbial community that is less efficient in decomposing litter, especially in pastures and *Eucalyptus* plantations.

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Supplementary Material

Table S1. Study sites information on location, age at sampling time, previous land use/cover type, and fertilization of each target land use/cover.

Site name/ Municipality	**Land cover or use**	**Geographical coordinates**	**Age in Aug/2015**	**Previous land use/cover**	**Fertilization**
Pirassununga/ Pirassununga	Cerradão	21° 56' 35" S; 47° 28' 26" W	original	Cerrado	None
	Pasture - *Urochloa decumbens*	21° 56' 42" S; 47° 28' 11" W	> 20 years	Corn and soybean	N-P-K 20-20-20 + Zn and Cu, at establishment
	Silviculture (*Eucalyptus saligna/ E. Urophylla*)	21° 58' 10" S; 47° 28' 25" W	16 years	Pasture	N-P-K 6-30-6 at establishment; casual local thinning
Santa Terezinha/ Bauru	Cerradão	22° 18’50" S; 49° 00' 13" W	original	Cerrado	None
	Pasture (*Urochloa decumbens*)	22° 18' 52" S; 48° 58 29" W	13 years	Cerrado	N-P-K yearly
	Siulviculture (*E. urophylla/ E. grandis/ E. Urograndis*)	22° 19' 35" S; 48° 58' 21" W	12 years	Cerrado	N-P-K 6-30-6 (January/2011); coppice at 10-12 years
Jardim/Bauru	Cerradão	22° 20' 45" S; 49° 00' 48" W	original	Cerrado	None
	Pasture (*Urochloa decumbens*)	22° 21’ 45" S; 49° 01' 34' W	> 20 years	Fruit (mango)	None
	Silviculture (*Eucalyptus urophylla*)	22° 13’ 38" S; 49° 12' 01" W	8 years	Pasture	N-P-K 13-13-13 at establishment; N-P-K 20-5-20 twice after planting

Table S2. Soil particle size distribution (Bouyoucos densimeter method; Camargo *et al*. 1986), pH (mean values; n=3) at the 0-10 cm depth and soil bulk density (mean values; n=3) from 0-5 and 30-35 cm depth for the study sites and target land uses /cover (LULC).

Site	LULC	Coarse Sand (%)	Fine Sand (%)	Total sand (%)	pH	Depth* (cm)	Bulk Density ($g.cm^{-3}$)
Pirassununga	Cerrado	51.1	43.7	94.7	5.19	0-5	1.13
						30-35	1.26
	Pasture	51.0	43.0	94.0	5.93	0-5	1.06
						30-35	1.38
	Eucalyptus	42.1	40.0	82.1	5.86	0-50	1.14
						30-35	1.11
Sta. Terezinha	Cerrado	53.4	39.7	93.1	5.13	0-5	1.29
						30-35	1.37
	Pasture	58.0	33.0	91.0	5.52	0-50	1.31
						30-35	1.37
	Eucalyptus	50.5	37.3	87.8	4.95	0-5	1.36
						30-35	1.30
Jardim	Cerrado	48.2	36.7	85.0	5.38	0-5	1.02
						30-35	1.20
	Pasture	51.8	34.4	86.2	5.75	0-5	1.18
						30-35	1.30
	Eucalyptus	46.3	35.2	81.5	5.60	0-5	1.42
						30-35	1.44

*Sampled depths for bulk density.

Table S3. Soil P, N and soil organic matter (SOM) concentrations for different LULC and five depths (0-10, 10-30, 30-50, 50-100, 100-200 cm). (Means ± Standard Error, n=3).

LULC	Depth (cm)	SOM (%)*	N ($g.kg^{-1}$)*	P ($mg.kg^{-1}$)*
Cerrado	10	1.114 ± 0.149	0.781 ± 0.067	5.735 ± 0.302
	30	0.948 ± 0.110	0.493 ± 0.023	3.806 ± 0.233
	50	0.900 ± 0.080	0.305 ± 0.023	2.843 ± 0.178
	100	0.686 ± 0.059	0.224 ± 0.023	2.097 ± 0.339
	200	0.764 ± 0.258	0.157 ± 0.028	1.656 ± 0.036
Pasture	10	1.082 ± 0.137	0.623 ± 0.192	7.102 ± 1.101
	30	0.925 ± 0.113	0.490 ± 0.138	4.435 ± 0.390
	50	0.815 ± 0.075	0.411 ± 0.121	3.378 ± 0.037
	100	0.688 ± 0.214	0.327 ± 0.103	2.674 ± 0.173
	200	0.473 ± 0.134	0.171 ± 0.010	2.229 ± 0.171
Eucalyptus	10	1.049 ± 0.148	0.704 ± 0.256	9.046 ± 2.098
	30	0.859 ± 0.159	0.545 ± 0.178	5.413 ± 0.737
	50	0.824 ± 0.056	0.385 ± 0.127	5.406 ± 0.545
	100	0.843 ± 0.123	0.267 ± 0.082	3.304 ± 0.373
	200	0.461 ± 0.160	0.212 ± 0.069	2.639 ± 0.524

*Results were averaged from the seven sampling points established in each site (Pirassununga, Sta. Terezinha and Jardim) for the 0-10 and 10-30 cm depth, and from four sampling points for the 30-50, 50-100 and 100-200 cm depth, all collected in the dry and wet seasons.

CHAPTER 2

The conversion of Brazilian woodland savanna (*cerradão*) to pasture and *Eucalyptus* plantations reduces soil nitrogen stocks and mineralization

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Abstract

The Brazilian savanna (Cerrado) has been extensively converted to croplands, pastures and forestry plantations, which may have caused critical changes in soil functioning, nutrient cycling, and thus in soil fertility. We assessed the impact of the woodland cerrado (*cerradão*) conversion into pastures and *Eucalyptus* plantations on the concentrations and stocks of total nitrogen (N), ammonia (NH_4-N) and nitrate (NO_3–N), and on the potential for nitrogen mineralization, quantified through the rates of potentially mineralizable nitrogen (PMN) and soil urease activity, in the first 200 cm of the soil profile. We also investigated the relative contribution of seasonal variation to the overall variability in soil N pools and mineralization potential. All the N pools and rates assessed sharply decreased from 10 to 50 cm depth, and barely changed below 50 cm. Inorganic forms of nitrogen (NH_4-N and NO_3-N) and PMN were the N-variables most significantly affected by land use conversion, with overall decreases in both land uses compared to native Cerrado, and lower values associated to *Eucalyptus* plantations. The average stock of inorganic N (NH_4-N + NO_3-N) in the top 30 cm of Cerrado soils was 68 kg ha^{-1}, with 44 kg ha^{-1} as NH_4-N and 24 kg ha^{-1} as NO_3-N. The conversion of Cerrado to pasture resulted in an overall 20% reduction of the inorganic N stock in the top 30 cm of the soil profile. The conversion to *Eucalyptus* decreased NH_4-N stocks by 30 % and increased NO_3-N stock by 16 %. Both types of conversions reduced PMN rates per unit area by one third, which represents a reduction of ∽2 Mg ha^{-1} y^{-1}. These results show negative impacts in nitrogen pools and cycling processes resulting from the cerradão conversion into pastures and *Eucalyptus* plantations under usual management, providing relevant information for land use planning of the Brazilian Cerrado areas.

Key words: Cerradão, *Eucalyptus* plantation, land use change, nitrogen cycling, potential mineralizable nitrogen, soil ammonium, soil nitrate, urease activity, *Urochloa* pasture.

Introduction

Land conversion from native ecosystems to agriculture and silviculture commonly results in important changes in biodiversity, ecosystem functioning and services provision (Foley *et al.*, 2005). Carbon and nutrient cycling are supporting ecosystem services that depend on plant-soil interactions and feedbacks, and therefore can be largely altered by changes in the land cover type (Lavelle *et al.*, 2005). For example, changes in vegetation structure and composition can modify the quality and quantity of litter, root exudates and root debris, leading to changes in the amount and quality of soil organic matter (Del Galdo *et al.*, 2003; DeGryze *et al.*, 2004). Land conversion may also lead to changes in water cycling and microclimatic conditions, and in the structure and activity of the soil microbial community, which are essential control factors of the biogeochemical cycling of carbon and nutrients (Scott and Binkley, 1997; Burket and Dick, 1998; Sotta *et al.*, 2008; Spera *et al.*, 2016). However, the actual impacts of land conversion in the provision of these fundamental supporting services may vary widely, depending on the natural ecosystem being transformed and the resulting new land use (McGrath *et al.*, 2001). This calls for research that helps to understand and quantify these impacts for the major sources of new agricultural land, pastures, and forestry plantations.

Tropical forests and woodlands are being transformed at very high rates in recent decades, particularly in forest-rich countries with low production costs such as Brazil, Malaysia, and Indonesia (Gibbs *et al.*, 2010). In Brazil, the remaining native primary vegetation area ranges from 15% in the Atlantic Forest region to 80-85% in the Amazon and Pantanal regions, with the Cerrado, Caatinga, and Pampa biomes retaining only around 50% of their original extent (Ribeiro *et al.*, 2009, Lapola *et al.*, 2014, Beuchle *et al.*, 2015, Grecchi *et al.*, 2015). Despite some successful efforts to reduce deforestation (Gibbs *et al.*, 2015), the conversion of native vegetation into agro-pastoral land-use is still very important in most Brazilian biomes, particularly within the Cerrado region (Lambin *et al.*, 2013, Noojipady *et al.*, 2017).

The Cerrado biome comprises a gradient of grassland-savanna-woodland physiognomies (Oliveira-Filho and Ratter, 2002), extended over an area of nearly 2 million km^2 in Brazil, and is considered to be one of the world's biodiversity hotspots (Myers *et al.*, 2000; Klink and Machado, 2005). Until the 1960s, Cerrado was exploited mainly for cattle ranching, which still is by far the dominant land use in the region. However, large-scale mechanized agriculture, forestry and intensive livestock farming essentially for producing commodities (Klink and Moreira, 2002; Lapola *et al.*, 2014) are increasingly replacing the natural or semi-natural pasturelands and expanding into native ecosystems, yet with strong geographic variation within the Cerrado region (Espírito-Santo *et al.*, 2016). Recent agricultural expansion has been concentrated in the northern part of the biome (known as Matopiba) that became a new agricultural frontier (Spera *et al.*, 2016; Noojipady *et al.*, 2017). In the state of São Paulo, less than 7% of Cerrado original area remained in the early 2000s (Durigan *et al.*, 2007), mainly under the woody physiognomies (*cerrado denso* and *cerradão*, *sensu* Ribeiro and Walter, 2008) and scattered as small fragments (Durigan *et al.*, 2007; Rodrigues *et al.*, 2008).

In the natural ecosystems, nutrient cycling is regulated through complex mechanisms and feedbacks across multiple scales that balance inputs and outputs, nutrient release and use, and thus limit net nutrient losses from the system (Lavelle *et al.*, 2005). Land conversion to cropland, planted pasture or silviculture often leads to imbalances in nutrient cycling due either to excessive nutrient inputs that cannot be retained and are ultimately transferred to aquatic ecosystems or lost as increased gas emissions (Verchot *et al.*, 1999; Howarth *et al.*, 2000), or to nutrient depletion in areas where nutrient content of the harvest (grain, timber, livestock) exceeds nutrient inputs. The latter is common in large areas of South America characterized by low soil fertility and limited use of fertilizers. As in most old, red soils of tropical South America, fertility is inherently low in the Cerrado soils, and land productivity is strongly limited by nitrogen (N) availability (Bustamante *et al.*, 2006). The conversion of the Cerrado natural areas to agricultural or silvicultural uses could therefore lead to lower N availability and declining overall fertility, yet it could be temporarily counterbalanced to greater or lesser extent by fertilization inputs. However, despite the importance of land conversion in Cerrado region, and the potential undesirable consequences for soil functioning, there has been little evaluation of land-use change impacts in N cycling in this biome. We aim to lessen this knowledge gap by investigating the effects on N availability and mineralization potential of the conversion of native Cerrado areas into pastures and *Eucalyptus* plantations, in several areas of São Paulo state. The target land uses represent contrasting nutrient inputs, as most pastures in Brazil are not (or little) fertilized (Groppo *et al.*, 2015), while *Eucalyptus* plantations commonly receive fertilization at planting, and may receive additional fertilization afterwards (Barros *et al.*, 2000).

In Brazil, *Eucalyptus* plantations cover more than 5.5 million ha, most of them in the Cerrado region (IBA 2015). Very few studies have assessed so far the impacts of this conversion in soil N pools and dynamics, reporting either no changes, small reduction or small increase in soil N stocks (Maquere *et al.*, 2008; Pegoraro *et al.*, 2011). Conversely, for the Cerrado-to-pasture conversion, previous studies reported a decrease in soil nitrogen content and stocks in non-fertilized pastures as compared with the original native Cerrado (e.g., Guareschi *et al.*, 2012; Groppo *et al.*, 2015), yet increased soil nitrogen has been also reported (Lilienfein and Wilcke, 2003; Maquere *et al.*, 2008). Although N mineralization and nitrification rates have been reported to be higher in intact tropical forests than in agricultural sites (Piccolo *et al.*, 1994; Reiners *et al.*, 1994; Neill *et al.*, 1999), forest conversions to croplands and pasturelands generally cause a rise in soil temperature, which stimulates biological activity and may result in increased N mineralization and nitrification (Piccolo *et al.*, 1994). Land use change can also affect the activity of soil urease, which is involved in N mineralization and can be largely affected by changes in the quantity and quality of organic inputs that follow deforestation (Salam *et al.*, 1998; Kizilkaya and Dengiz, 2010). The temporal (seasonal and interannual) and spatial (between and within sites) inherent variation in soil and climatic conditions, as well as management differences, may contribute to the lack of consistency found in the few studies addressing the effects of Cerrado conversion to pastures and *Eucalyptus* plantations on N-cycling. Furthermore, variations in plant nutrient uptake and organic matter inputs resulting from land cover changes may exert either synergistic or opposite effects on N-cycling, contributing with additional source of variation to the overall land conversion effect.

We aimed to assess the overall impact of the conversion of Cerrado to pastures and *Eucalyptus* plantations on N cycling and pools, considering a wide range of spatio-temporal variability due to soil depth, site conditions, and seasonal variation. For three sites in São Paulo state, each of them including remnants of native woodland cerrado (*cerradão*) and areas converted to pasture and *Eucalyptus* plantations, we investigated the effects of land conversion on the concentration and stocks of total N and the inorganic NH_4-N and NO_3–N forms, and on the potential for nitrogen mineralization, quantified through the rates of potentially mineralizable nitrogen (PMN) and soil urease activity. Given the large depth of most Cerrado soils, which hold an extraordinarily high belowground biomass under natural vegetation (Coutinho, 1990; Castro and Kauffman, 1998), we investigated how the impact of land conversion may change with soil depth throughout the first 2 meters of the soil profile. Finally, we investigated the relative contribution of seasonal variation to the overall variability in soil N pools and mineralization potential.

Materials and methods

Study area

This research was carried out in a region originally covered by the Cerrado biome in São Paulo state, Brazil, at Pirassununga and Bauru municipalities. The regional climate is tropical, humid, with wet summers and dry winters. Annual average rainfall ranges between 1300 mm (Bauru weather station; 22º19' S – 49º02' W; 1945-2001 rainfall series) and 1420 mm (Pirassununga weather station; 21º58' S – 47º28' W; 1954-1999), and the rainy season goes from October to March (DAEE, Department of Water and Electric Energy of São Paulo state).

We focused our study on the woodland cerrado physiognomy, the *cerradão*, as this physiognomy tends to dominate among the Cerrado remnants in São Paulo State and other Cerrado areas at the expense of more open physiognomies due to fire suppression and decreasing grazing (Pivello, 2011; Durigan and Ratter, 2016; Abreu *et al.*, 2017). We used three study sites: Pirassununga (Pirassununga municipality), Santa Terezinha and Jardim (both in Bauru municipality). Each site included one fragment of each target land cover and use (Supplementary Material, Table S1): native Cerrado (*cerradão* physiognomy), beef cattle pasture (of planted *Urochloa decumbens*, introduced African grass species), and *Eucalyptus* plantation (*Eucalyptus saligna*, *E. urophylla*, *E. grandis*, *E. urograndis*). The three sites were on flat or gently slope terrains (slope of 0 to 5%), and soils were sandy and dystrophic Red Latosol and Dark-Red Latosol soil type (Santos *et al.*, 2013) , with more than 80% of sand, prevailing the coarse sand, and pH varying from 4.95 to 5.93 (Supplementary Material, Table S2). Fertilization of the target pastures was nil or consisted of N-P-K addition only at establishment. The *Eucalyptus* plantations received liming and N-P-K at establishment and, depending on the site, none, one or two additional inputs after planting (Table 1S).

Experimental design and sampling

The study followed a randomized block design, with two fixed factors: LULC (land use/ land cover: pasture, *Eucalyptus* plantation and Cerrado) and soil depth (0-10, 10-30, 30-50, 50-100, and 100-200 cm), and one random factor: site. For each target

LULC and site, we collected soil from seven sampling points distributed at regular distances along a 140-m long transect, with four of them being sampled during the dry season in 2015 (August-October/2015) and three of them, spatially interspersed between the previous ones, being sampled during the wet season in 2016 (April-May/2016). We sampled soils with a 17-cm diameter corer at depths of 0-10 and 10-30 cm from all seven sampling points, plus at depths of 30-50, 50-100, and 100-200 cm from four of the sampling points (two per season). Soil samples were sieved at ≤ 2mm, and separated in two sub-samples: one field moist-soil sample that was stored at 4ºC in plastic bags until analyses, and one dry-soil sample that was air-dried and stored until analyses. Additional soil samples were taken at 0-5 and 30-35 cm soil depth (three undisturbed samples per depth, LULC and site) and dried at 105°C for determination of the soil bulk density (volumetric ring method; EMBRAPA 1997) for the uppermost soil layers.

Soil analyses

Soil ammonium (NH_4–N) and nitrate (NO_3–N) nitrogen were quantified by the salicylate/nitroprusside and salicylic acid colorimetric methods, respectively (Cataldo *et al.*, 1975; Baethgen and Alley, 1989). The extraction procedure consisted of 1-hour mechanically shaking 10 grams of moist soil and 30 ml of 0.5 M K_2SO_4, and further filtering of the solution through Whatman No. 2 filter paper. Potentially mineralizable nitrogen (PMN) was determined by soil incubation at 40º C for 10 days; after incubation PMN was extracted by 0.625M K_2SO_4, and determination of NH_4–N was made by the salicylate/nitroprusside method. Urease activity was determined by quantifying the ammonium released into the incubation solution of 0.5 g of dry soil with urea solution at 37°C for two hours (Kandeler and Gerber, 1988). We analysed soil total N in dry soils, using the microKjeldahl digestion procedure (following Raij *et al.*, 1987). In dry soils from the first sampling period (dry season), we analysed soil organic carbon (SOC) by sodium dichromate oxidation in H_2SO_4 and colorimetric quantification (Nelson and Sommers, 1982); we then calculated the soil C:N ratio using total N and SOC data from the dry season. Nitrogen stocks were calculated by considering the N-variable concentration (g kg^{-1}), soil bulk density ($g.cm^{-3}$), and the depth of the soil layer (cm) (Rangel and Silva, 2007; Groppo *et al.*, 2015). Bulk density was determined by the volumetric ring method (Blake and Hartge, 1986; EMBRAPA 1997) from undisturbed soil cores sampled from 0-5 and 30-35 cm soil depths; deeper soil samples did not result in accurate volumetric estimations and were disregarded. Accordingly, nitrogen stocks were only estimated for the uppermost soil layers.

Data analyses

For each variable and soil depth, the data from the various sampling points on each sampling transect were averaged, so that a single value per depth, LULC, site and season, integrating the within-site spatial and temporal variation of each target variable was used for the analysis of LULC and soil depth effects and interactions. These data were analysed using a mixed-effect analysis of variance, with LULC (pasture, *Eucalyptus* and Cerrado), and soil depth as fixed effects, and site as random effect. Cumulative soil N stocks and mineralization rates per unit area were calculated for fixed depths by multiplying the soil nitrogen concentration at the designated depth and the soil bulk density. Soil bulk density values from 0-5 and 30-35 cm soil samples were used to estimate stocks for 0-10 and 30-50 cm soil layers, respectively; averaged bulk density values from 0-5 and 30-35 cm soil samples were used to estimate stocks for the 10-30 cm soil layer. For each depth, within-site differences in these N stocks and mineralization rates between Cerrado and either pasture or *Eucalyptus* plantations (i.e. significant deviations of these differences from zero) were analysed using two-tailed Student's one-sample t-tests. Relationships between soil N variables were explored by Pearson correlations. To visualize how LULC, between- and within-site spatial variation, and seasonal variation contributed to the overall heterogeneity of soil N pools, we used a Principal components analysis (PCA) performed on NH_4-N, NO_3-N, PMN and urease activity data from the uppermost soil layers (averaged values from depth of 0-10 and 10-30 cm), plotting each sampling point against the two main axes of the PCA. Statistical procedures were performed using SPSS 20.0 for Windows.

Results

For all sites and LULC, all soil N variables followed a decreasing pattern with soil depth (Figures 1 and 2), exhibiting a sharp reduction from 10 to 50 cm depth and barely changing below 50 cm. The effect of soil depth was highly significant for all N variables (Table 1), yet the depth-dependent variation was more pronounced for urease activity and PMN than for the NH_4-N, NO_3-N, and total N pools. LULC effect was significant for NH_4-N, with higher values in Cerrado than in pastures or *Eucalyptus* plantations, and marginally significant for NO_3-N, with lower values in pastures and similar values in Cerrado and *Eucalyptus* (Figure 1). Total N, PMN, and urease did not show any significant effect of LULC, yet the interaction between LULC and site was highly significant for total N and PMN (Table 1), with two sites showing higher values in Cerrado and one site (Pirassununga) showing no variation between land uses for these two variables (Figures 1 and 2). Significant interactions between LULC and soil depth (Table 1) reflect the different depth profiles of N variables in Cerrado and the two other land uses, with Cerrado showing much higher values at 0-10 cm depth and a sharper decrease with depth than pastures and *Eucalyptus*. The soil C:N ratio significantly increased with soil depth (F=5.4; *P*=0.022), with average values for the three LULCs varying from 11.5 ± 0.3 at 0-10 cm depth to 15.5 ± 1.2 at 30-50 cm depth, and greatly increasing up to 39.1 ± 3.1 at 100-200 cm depth. The C:N ratio did not vary significantly with LULC (F=1.3; *P*=0.365), with average C/N values at 0-50 cm of 14.1 ± 1.7, 12.1 ± 0.5, and 13.5 ± 1.6 for Cerrado, *Eucalypus* and pasture, respectively; yet it showed a marginally significant interaction between LULC and site (F=2.4; *P*=0.095), and a significant interaction between site and soil depth (F=7.1; *P*=0.001).

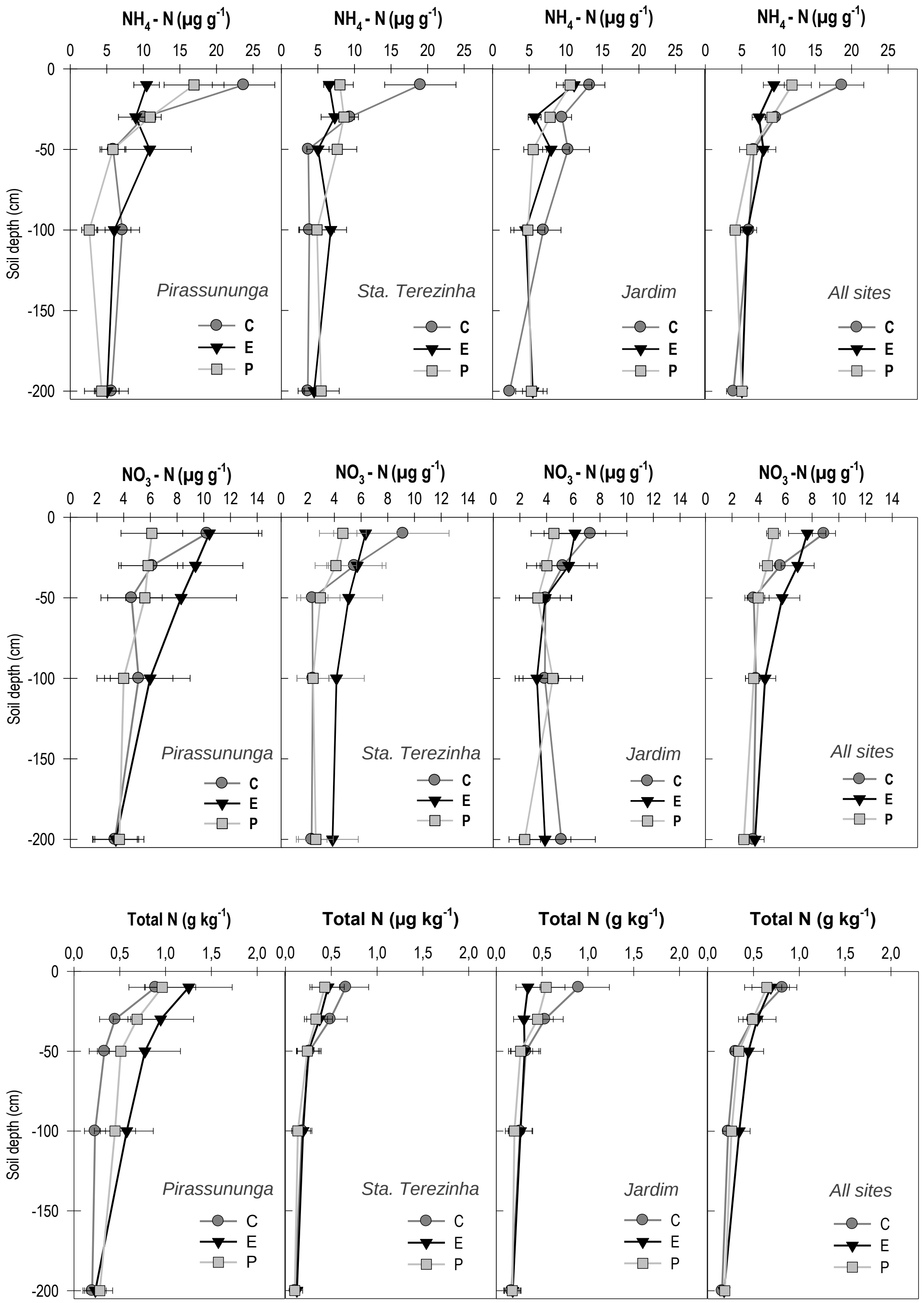

Figure 1. Variation in NH4-N (top panel), NO3-N (medium panel), and total N (bottom panel) pools with soil depth for the three target LULC (pasture Eucalyptus and Cerrado) for each study site (Pirassununga, Sta. Terezinha, and Jardim) and for the average of the three sites (all-sites). Values are means ± standard error (for individual sites, n = 7 for 0 -10 and 10-30 cm soil depth, and n = 4 for 30-50, 50-100 and 100 -200 cm soil depth).

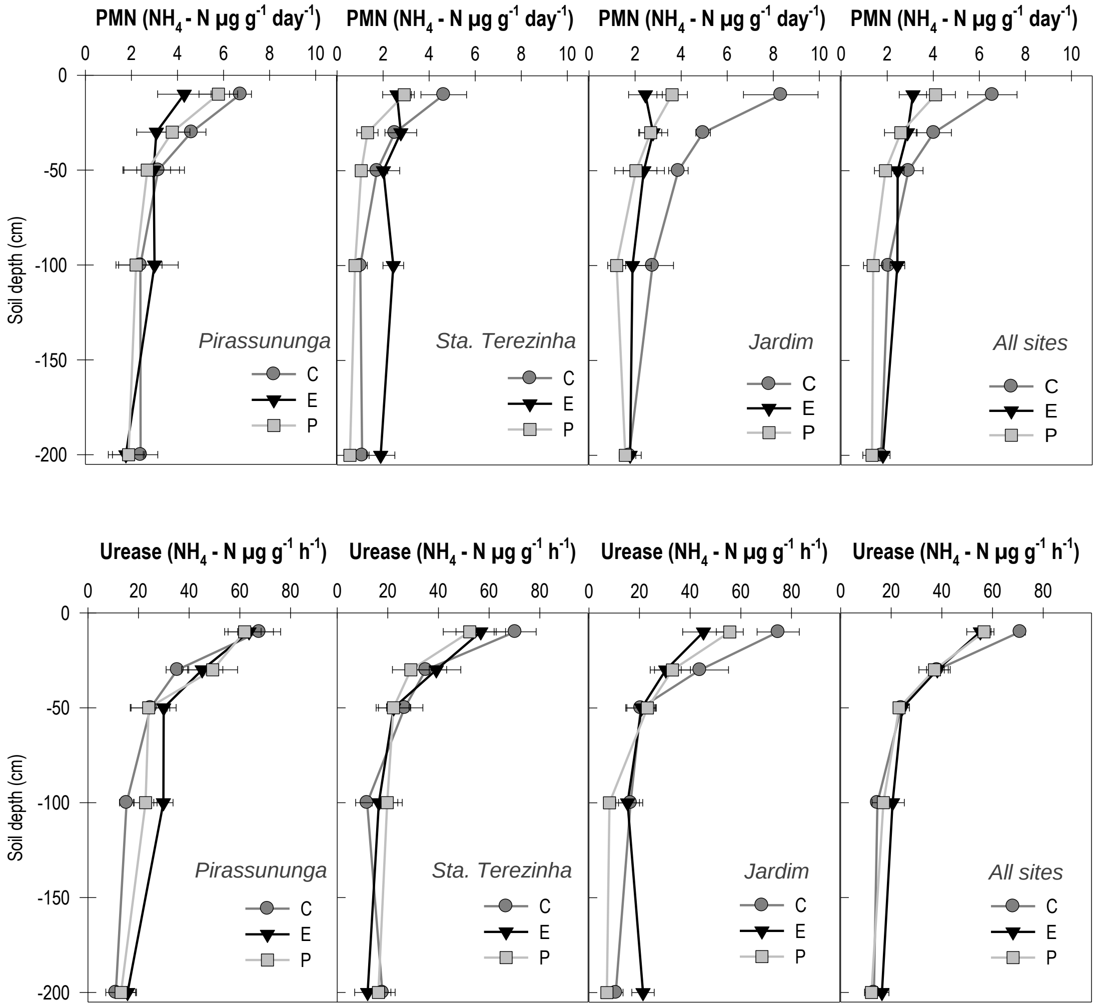

Figure 2. Variation in potentially mineralizable nitrogen (PMN, top panel) and soil urease activity (bottom panel) with soil depth for the three target LULC (pasture, Eucalyptus and Cerrado) for each study site (Pirassununga, Sta. Terezinha, and Jardim) and for the average of the three sites (all-sites). Values are means ± standard error (for individual sites, n = 7 for 0 -10 and 10-30 cm soil depth, and n = 4 for 30-50, 50-100 and 100 -200 cm soil depth).

Table 1. Results of mixed-effects Analysis of Variance on N pools (NH4-N, NO3-N, and total N) and mineralization rate indicators (PMN and urease activity); F statistic (and P value) for main and interaction effects. LULC: land use / land cover. In bold: significant effects; in bold and italics: marginally significant ($p \leq 0.1$) effects.

Effect	**NH_4-N**	**NO_3-N**	**Tot N**	**PMN**	**Urease**
LULC	F=19.6	F=6.7	F=0.1	F=3.0	F=0.8
	(**0.009**)	(***0.052***)	(0.870)	(0.160)	(0.518)
Soil depth (D)	F=17.5	F=15.2	F=20.5	F=47.8	F=251.8
	(**0.001**)	(**0.001**)	(<**0.001**)	(<**0.001**)	(<**0.001**)
Site (S)	F=16.6	F=7.4	F=3.8	F=4.0	F=2.2
	(0.681)	(**0.028**)	(***0.099***)	(0.108)	(0.290)
LULC x D	F=2.7	F=3.3	F=1.2	F=8.0	F=2.3
	(**0.043**)	(**0.020**)	(0.375)	(<**0.001**)	(***0.071***)
LULC x S	F=0.1	F=2.1	F=9.6	F=8.7	F=2.3
	(0.968)	(0.123)	(<**0.001**)	(<**0.001**)	(0.104)
D x S	F=1.0	F=1.9	F=2.2	F=1.2	F=0.5
	(0.451)	(0.134)	(***0.085***)	(0.376)	(0.827)

The average within-site losses and gains in N stocks and mineralization rates due to the conversion of Cerrado to pastures and *Eucalyptus* plantations showed a consistent pattern of losses in the uppermost soil layers, with significant decrease in NH_4-N stock at 10-30 cm, and marginally significant decrease of PMN rate at 0-10 cm depth for the conversion to *Eucalyptus*, and marginally significant decrease in NO_3-N stock at 0-10 cm depth, PMN rate at 0-10 and 10-30 cm depth, and urease activity per unit area at 0-10 cm depth for the conversion to pasture (Figure 3). The soil below 30 cm depth showed no relevant changes, with the exception of NO_3-N, which exhibited a marginally significant increase at 30-50 cm depth for the conversion to *Eucalyptus*. Changes in total N stocks were not significant at any depth (Figure 3). Considering the pooled data for the first 30 cm of the soil profile (Table 2), the conversion of Cerrado to pasture resulted in an overall reduction in the stocks of inorganic N (NH_4-N + NO_3-N) of ∽20 %, affecting both NH_4-N and NO_3-N similarly. The conversion to *Eucalyptus* decreased NH_4-N stocks by 30 % and increased NO_3-N stock by 16 %, resulting in an overall ∽14 % (∽10 kg ha^{-1}) decrease in inorganic N. Both types of conversions reduced PMN rates per unit area by one third, which represents a reduction of ∽2 Mg ha^{-1} y^{-1}. Urease amounts were also decreased ∽10% in pastures.

Table 2. Soil (0-30 cm depth) N-stocks for NH4-N, NO3- N (kg ha-1) and total N (Mg ha-1); PMN and urease activity rates per area unit (kg ha-1 year-1); and soil bulk density (g cc-1) for Cerrado (C), Eucalyptus (E) and pastures (P). Average values ± 1 standard error (n = 3 study sites).

N-variable	**Cerrado**	***Eucalyptus***	**Pasture**
NH_4-N (kg ha^{-1})	44.9 ± 5.2	30.9 ± 1.3	36.7 ± 4.0
NO_3-N (kg ha^{-1})	23.9 ± 2.4	27.3 ± 2.9	17.7 ± 1.6
Inorganic N (kg ha^{-1})	68.8 ± 7.6	58.2 ± 3.8	54.4 ± 5.6
Total N (Mg ha^{-1})	2.1 ± 0.1	2.2 ± 0.7	2.0 ± 0.4
PMN (Mg $ha^{-1}year^{-1}$)	6.2 ± 0.8	4.2 ± 0.1	4.1 ± 0.8
Urease (Mg $ha^{-1}year^{-1}$)	63.0 ± 2.4	61.6 ± 3.3	56.2 ± 2.6
Bulk density* (g cm^{-3})	1.21 ± 0.06	1.30 ± 0.09	1.27 ± 0.04

*Average bulk density from 0-5 and 30-35 cm soil layers.

With the only exception of PMN and NO_3-N, all other N-variables were correlated to each other (Supplementary material; Table S3), yet there exhibited different sensitivity to the various factors and sources of heterogeneity. Figure 4 illustrates the contribution of LULC, season and between- and within-site spatial variability to the overall variation N-variables for the top 0-30 cm according to the PCA ordination of the sampling points. The first axis PC1 was mostly and positively correlated with NO_3-N and urease activity, and captured the variation due to season, with a trend towards higher values for the rainy season. The second axis PC2, which was positively correlated with NH_4-N, captured the variation associated to LULC, with higher values for Cerrado and lower values for *Eucalyptus* that was most evident for the dry season.

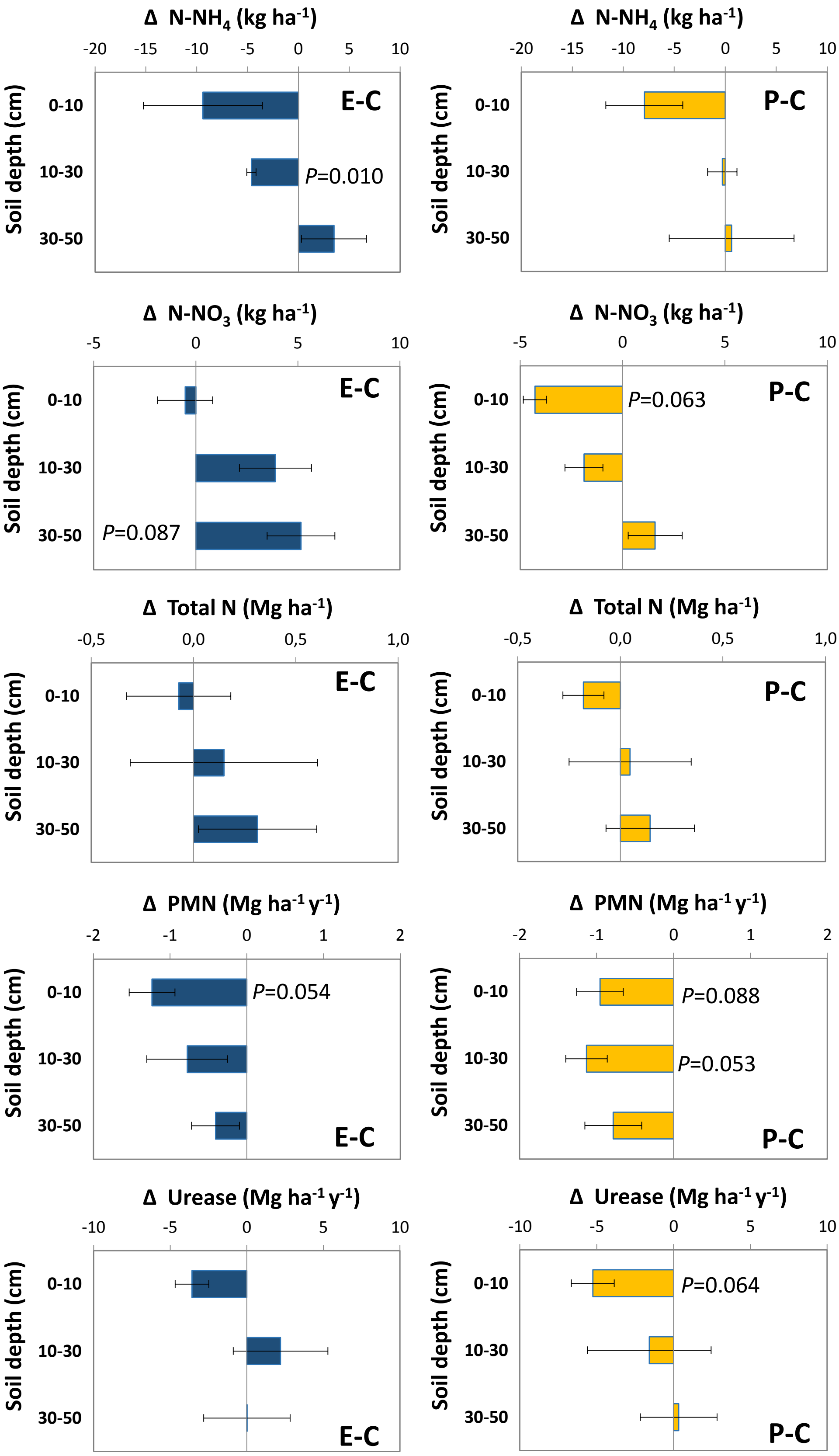

Figure 3. Average changes (gains and losses) for NH4-N, NO3- N (kg ha-1), and total N (Mg ha-1) stocks, and PMN and urease activity rates per area unit (kg ha-1 y-1), at 0-10, 10-30, and 30-50 cm depth for the conversion of Cerrado into Eucalyptus (E-C) and pastures (P-C). Negative values represent net losses and positive values represent net gains (Mean ± standard error; n = 3). Marginally significant P values indicated.

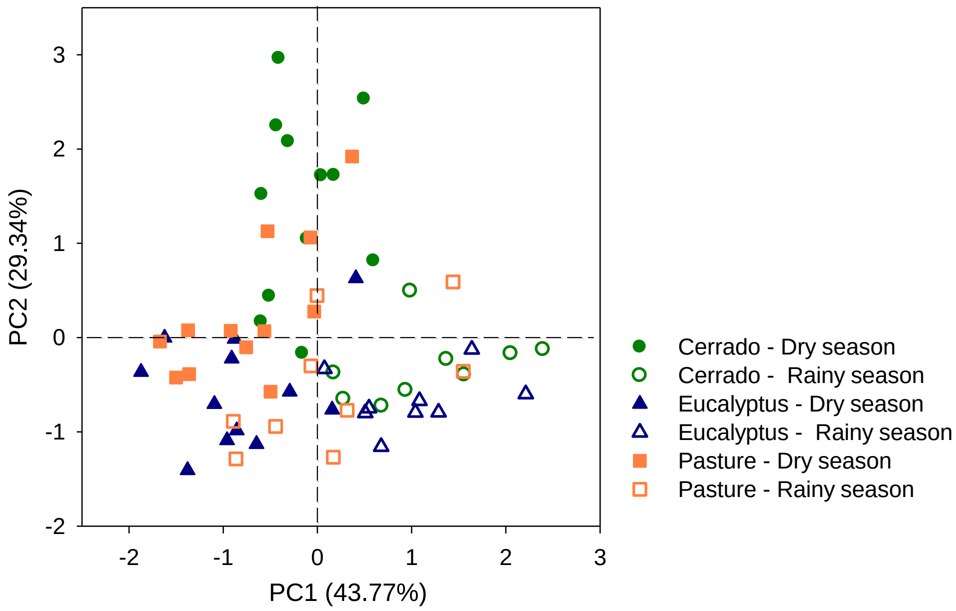

Figure 4. PCA-based ordination of sampling plots (symbols) for each LULC (Cerrado, Pasture and Eucalyptus) and sampling season (dry, rainy) based on linear combinations of NH4-N, NO3-N, PMN, and urease activity values.

Discussion

The conversion of Cerrado (cerradão physiognomy) into pastures and *Eucalyptus* plantations resulted in an overall decrease in the concentrations and stocks of inorganic N forms (NH_4-N and NO_3-N) and also in the potential mineralization of nitrogen, yet only significant for the uppermost 30 cm of the soil profile. Despite a strong spatio-temporal variation in soil functioning that resulted from seasonal and site differences, our findings reveal a clear and relatively fast nutrient depletion pattern associated to land conversion in the Brazilian Cerrado.

The concentration and distribution pattern of soil nitrogen-related variables was characterized by higher values at the topsoil layer (0–30 cm depth) in all LULC, with a sharp decrease below 30 cm. Similar results were observed by other studies (Groppo *et al.*, 2015; Kizilkaya and Dengiz, 2010). The higher values of soil nitrogen variables in the uppermost layer could be explained by a higher organic matter content and microbial activity in that layer (Kizilkaya and Dengiz, 2010), as the most common factors affecting soil nitrogen cycling are substrate quality and quantity, size of microbial biomass pool, availability of soil carbon, and soil moisture content (Sotta *et al.*, 2008).

Comparing LULC, the values of NH_4-N, PMN and urease in the most superficial layers tended to follow the pattern: Cerrado > pasture > *Eucalyptus* plantation. These variables are very related to microbial activity. In the natural environment of Cerrado, biotic (organic matter quality) and abiotic (lower soil density, that indicates less compacted soil and more aeration) conditions contribute to microbial and microfauna activity. Still, *Eucalyptus* plantations can negatively affect soil organic matter (SOM) decomposition and the nutrient cycling (especially for C and N) due to the presence of antibacterial substances (Bruna *et al.*, 1989) and high litter C:N ratios (Pillon *et al.*, 2011), which can reduce the bacteria population (including nitrifying bacteria). In fact, the lowest values of NH_4-N and PMN are found in the *Eucalyptus* plantations. Other studies reported negligible N inputs by N_2 biological fixation in *Eucalyptus* plantations under Cerrado soils (e.g., Laclau *et al*., 2010). PMN and urease values remained relatively higher deeper in the Cerrado-Jardim soil, which is the most preserved of the studied cerrados, indicating higher N mineralization potential in that soil. A distinct pattern was verified in Pirassununga concerning NO_3-N, where higher concentrations along the entire soil profile were registered in the *Eucalyptus* plantation compared to Cerrado and pasture. Probably drawn by NO_3-N values, total N values showed this same pattern in Pirassununga. The reason for those considerably higher values of NO_3-N are unknown, as soil conditions and management were very similar to the other *Eucalyptus* sites except for the fact that it was the oldest plantation (Tables S1 and S2). Nevertheless, Gama-Rodrigues *et al.* (2005) found a positive relationship between soil organic carbon (SOC) concentrations and NO_3-N in *Eucalyptus* plantations in São Paulo state. In fact, we verified higher soil organic carbon contents for the *Eucalyptus* plantation in Pirassununga (G.S. Brito *et al.*, in prep.), which could be due to less agricultural management in that site.

Previous studies including the Cerrado biome, such as the one by Guareschi *et al.* (2012) and Groppo *et al.* (2015), found important losses of total nitrogen in pastures compared to native vegetation, some associated to carbon loss in the new land use. In the 0-30cm soil layer, Groppo *et al.*, (2015) observed a reduction of total nitrogen stocks from 5.12 Mg ha^{-1} in native vegetation (Cerrado + Atlantic Forest+ grasslands), to about 3.9 $Mg.ha^{-1}$ in pastures. On the other side, Maquere *et al.* (2008) reported nitrogen gains in pastures settled in Cerrado lands, which increased with the pasture age, however, these results were in part due to soil compaction, and consequently, higher density. Nitrogen stocks at 0-30 cm depth varied form 2.2 $Mg.ha^{-1}$ in Cerrado to 2.4 to 2.5 $Mg.ha^{-1}$ in pastures. The same authors also compared the native Cerrado vegetation to *Eucalyptus* plantations and, for a 60-year *Eucalyptus saligna* stand, they observed a trend of smaller N stocks (2.0 $Mg.ha^{-1}$) compared to Cerrado, although not in the younger stands (6-10 years old). Pulrolnik *et al.* (2009) also found total nitrogen increases in Cerrado soils under pasture but decreases in soils under a 20-year-old *Eucalyptus urophylla* stand. In our case, the values for total nitrogen stocks at 0-30 cm soil layer in Cerrado (2.08 $Mg.ha^{-1}$), pasture (1.98 $Mg.ha^{-1}$) and Eucalyptus (2.18 $Mg.ha^{-1}$) were at similar magnitude as those found by Maquere *et al.*, (2008). We observed losses of total nitrogen in pastures, and more evident in the Jardim site. Still, unfertilized soils show an inverse relationship between age and nitrogen availability (Groppo *et al.*, 2015). This is the case of the pastures we sampled, which represent a common practice adopted in the Cerrado small farms for beef cattle production: old pastures, poorly managed and supporting a low stocking rate (around one animal per hectare) (Lapola *et al.* 2014), where the low density of animals is not able to replace by urine the nitrogen withdrawn by cattle feeding. In addition, cattle excreta are responsible for almost 50% of N losses in livestock pastures, which occur via volatilisation of NH_4^+ (Vallis 1985). Our results also show substantial decreases in the stocks of all nitrogen-related variables up to 50 cm soil depth in pastures compared to Cerrado, with the only exception of NO_3-N at 30-50 cm. D'Andréa *et al.* (2004) also found NH_4-N decreases in superficial layers of a *Urochloa decumbens* pasture in Cerrado. In our studied *Eucalyptus* stands, stocks of NH_4-N were much lower compared to Cerrado, probably due to a preferential absorption of the NH_4-N nitrogen form by *Eucalyptus* trees (Vale *et al.*, 1984). On the other hand, our results show higher stocks of NO_3-N below 10 cm (that resulted on higher total N stocks) related to Cerrado, that are probably due to fertilization.

Acidic and low fertile soils usually have the prevalence of NH_4-N over NO_3-N (Gama-Rodrigues *et al.*, 2005; Groppo *et al.*, 2015). Our results show NH_4-N dominance in all LULC up to 200 cm depth including the native Cerrado. Similarly, D'Andréa *et al.* (2004) reported NH_4-N dominance over NO_3-N up to 100 cm depth in native Cerrado and in a 15-year-old *Urochloa decumbens* pasture. Other authors have reported NH_4-N prevalence in *Eucalyptus* plantations on converted Cerrado soils (Gama-Rodrigues *et al.*, 2005) and also in tropical forests converted to pastures in Brazil (Matson *et al.*, 1990; Luizão *et al.*, 1992; Neill *et al.*, 1995) and in Costa Rica (Reiners *et al.*, 1994).

The N mineralization potential in the soils of the three LULC, expressed as urease activity, potentially mineralizable nitrogen, and amount of available forms of nitrogen

(NH_4-N, NO_3-N), was positively and strongly related with the total amounts of soil organic N and C. Similar results were reported by previous studies that recognized the importance of organic matter in the soil nitrogen dynamics (Recous *et al.*, 1995; Sotta *et al.*, 2008). A greater variation in the inorganic forms of nitrogen compared to PMN probably resulted from the between-site spatial and temporal heterogeneity in soil moisture and temperature, which largely affects the actual mineralization rates. Conversely to the inorganic forms of nitrogen, PMN is related to less variable soil properties, such as soil organic content and overall condition of the soil microbial community.

When all the nitrogen-related variables were jointly analysed, they showed clear changes due to the conversion of native vegetation into the target land uses assessed, with the changes affecting mostly the topsoil layers. The results from a PCA analysis showed larger differences in *Eucalyptus* plantation than in pastures regarding Cerrado soils, and this can be expected due to plant species functional types and physiological behaviour besides the agricultural management in the silviculture. *Eucalyptus* plantations have high N uptake and management includes the use of heavy machinery and a rotation system, which cause high soil disturbance, the input (by fertilization) of high amounts of chemicals (especially N, P, K and Ca) and also the output of great amounts of nutrients (through timber harvesting) can lead to nutrient decline in the soil (Laclau *et al.*, 2010) or accumulation in the lower soil layers, as observed here for NO_3-N. In addition, being *Eucalyptus* litter harder to decompose (Mendham *et al.*, 2004) soil microbial activity can be impaired, and N stocks affected. The results from the PCA also show higher soil microbial activity (through urease production) in the wet season, as well as NO_3-N accumulation as registered by Bustamante *et al.* (2006).

Our results highlight the great influence of common management practices in Brazil on soil N variables. If well-managed pastures usually result in higher amounts of N due to fertilization and urine (higher stocking rate), degraded low-productive pastures (without proper fertilization) result in soil N losses. Cerrado is the Brazilian region with the largest extent of livestock pastures (Lapola *et al.*, 2014), which are mostly under some degree of degradation (Klink and Machado, 2005; Costa *et al.*, 2006). Brazil also stays among the six largest producers of *Eucalyptus* pulp for the paper industry. Our data also show high N losses in *Eucalyptus* stands on Cerrado soils that may be associated to soil disturbance and compaction, high plant uptake and changes in litter quantity and quality, combined with local soil characteristics and climate. Still, according to Laclau *et al.*

(2010), *Eucalyptus* plantations benefits from the soil fertility from the previous land use/cover, and require increased inputs of nutrients in the long-term. Therefore, appropriate agricultural management and the constant monitoring of soil chemical properties are essential to maintain appropriate soil health and fertility.

Conclusion

The conversion of native Cerrado into pastures and *Eucalyptus* plantations resulted in substantial nitrogen losses in the upper soil layers. Poor agricultural management – the case of the studied pastures –, high soil disturbance that usually happens in silvicultures, associated to exotic vegetation with very different characteristics of the native one – such as for the *Eucalyptus* trees – may explain those losses. Climate seasonality influenced soil N variables.

Inorganic forms of nitrogen (NH_4-N and NO_3-N) and PMN were the N-variables most significantly affected by land use conversion, with overall decreases in both land uses compared to native Cerrado, and lower values associated to *Eucalyptus* plantations. The prevalence of NH_4-N over NO_3-N was registered up to 200 cm depth in all LULC, which is associated to low fertility and acidic soils.

All N variables decreased with depth, with higher values up to 30 cm. *Eucalyptus* plantation in Pirassununga site, which was the oldest stand, showed NO_3-N values higher than Cerrado for the entire soil profile, and no NH_4-N prevalence, probably due to the absence of a rotation system, the only management difference from the other *Eucalyptus* stands.

Urease activity also decreased in pastures and *Eucalyptus* plantations, with lower values in the last. Urease and NH_4-N reductions point to less favorable conditions for microbial activity and growth in the studied pastures and *Eucalyptus* stands. The most preserved Cerrado vegetation had the highest potential for mineralization also in deeper soils.

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Supplementary material

Table S1. Location, age at sampling time, previous land use/cover type, and fertilization of each target land use/cover at the study sites.

Site name/ Municipality	Land use / cover	Coordinates	Age	Previous land use/ cover	Fertilization
Pirassununga/ Pirassununga	Cerradão	21°56'35" S; 47°28'26" W	original	Cerrado	None
	Pasture - *Urochloa decumbens*	21°56'42" S; 47°28'11" W	> 20 years	Corn and soybean	N-P-K 20-20-20 + Zn, Cu, at establishment
	Silviculture *Eucalyptus saligna/ E. Urophylla*	21°58'10" S; 47°28'25" W	16 years	Pasture	N-P-K 6-30-6 at establishment
Santa Terezinha/ Bauru	Cerradão	22°18’50" S; 49°00'13" W	original	Cerrado	None
	Pasture *U. decumbens*	22°18'52" S; 48°58'29" W	13 years	Cerrado	N-P-K yearly
	Silviculture *E. urophylla/ E. grandis/ E. urograndis*	22°19'35" S; 48°58'21" W	12 years	Cerrado	N-P-K 6-30-6 at establishment and on January 2011
Jardim/Bauru	Cerradão	22°20'45" S; 49°00'48" W	original	Cerrado	None
	Pasture *U. decumbens*	22°21’45" S; 49°01'34' W	> 20 years	Fruit (mango)	None
	Silviculture *E. urophylla*	22°13’38" S; 49°12' 01" W	8 years	Pasture	N-P-K 13-13-13 at establishment; N-P-K 20-5-20 twice after planting

Table S2. Soil particle size distribution (Bouyoucos densimeter method; Camargo *et al.* 1986) and pH (mean values; n=3) at the 0-10 cm depth for the study sites and target land uses /cover.

Site	**Land Use**	**Coarse Sand (%)**	**Fine Sand (%)**	**Total sand (%)**	**pH**
Pirassununga	Cerrado	51.1	43.7	94.7	5.19
	Pasture	51.0	43.0	94.0	5.93
	Eucalyptus	42.1	40.0	82.1	5.86
Sta. Terezinha	Cerrado	53.4	39.7	93.1	5.13
	Pasture	58.0	33.0	91.0	5.52
	Eucalyptus	50.5	37.3	87.8	4.95
Jardim	Cerrado	48.2	36.7	85.0	5.38
	Pasture	51.8	34.4	86.2	5.75
	Eucalyptus	46.3	35.2	81.5	5.60

.

Table S3. Pair-wise Pearson's correlation coefficients (and *P* values) for ammonium nitrogen (NH_4-N), nitrate nitrogen (NO_3-N), potentially mineralizable nitrogen (PMN), total nitrogen (TN), soil urease activity (UR), and C:N ratio. Data are averages per depth, land use and site (n=45).

	NH_4-N	**NO_3-N**	**PMN**	**TN**	**UR**
NO_3-N	0,426 **(0,001)**				
PMN	0,651 **(< 0.001)**	0,168 (0,219)			
TN	0,631 **(< 0.001)**	0,613 **(< 0.001)**	0,591 **(< 0.001)**		
UR	0,574 **(< 0.001)**	0,400 **(0,002)**	0,686 **(< 0.001)**	0,737 **(< 0.001)**	
C:N	-0,378 **(0,004)**	-0,316 **(0,019)**	-0,343 **(0,010)**	-0,690 **(0< 0.001)**	**-0,502 (< 0.001)**

CHAPTER 3

Changes in carbon and nitrogen stocks after conversion of Brazilian woodland savanna (*Cerradão*) to pasture and silviculture

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Abstract

Native vegetation replacement by pasture and silviculture can result in clear changes on nutrients pools, such as C and N above and belowground. We aimed to assess effects of land use/land cover (LULC) change on C (above and belowground) and N (belowground) stocks, distribution, and overall balance after the conversion of woodland Cerrado ecosystems to livestock *Urochloa* pastures and *Eucalyptus* plantations in three landscapes (study sites) that included fragments of native Cerrado and both land uses. We estimated the total belowground C stock up to 50cm depth – composed of soil organic carbon stock (SOCS) + dissolved organic carbon stock (DOCS) + microbial biomass carbon stock (MBCS) + fine and coarse root C stock – and the overall aboveground C stock – litter + herbs/shrubs + tree C stocks –, as well as the overall N stock belowground up to 50cm – composed by total N stock + inorganic N stock (NH_4-N and NO_3–N) + potentially mineralizable nitrogen (PMN) stock + N root (fine and coarse) stock. LULC conversion resulted in C and N losses, and their redistribution among the system compartments. For Cerrado fragments, the mean total C stock (above + belowground) was 90.89 $Mg.ha^{-1}$, pastures showed 53% less, and *Eucalyptus* plantations increased 20%. Carbon was mostly stored belowground in pastures, and aboveground in *Eucalyptus* plantations, while Cerrado showed a balanced distribution above/belowground. Compared to native Cerrado, aboveground C stocks decreased 94% in pastures and increased 80% in *Eucalyptus* plantations; belowground, pastures decreased 19% and 25% in the overall C and N stocks respectively, with significant reductions in the arboreal C stock, MBCS, coarse root C and N stocks, NO_3-N stock and PMNS. *Eucalyptus* plantations decreased 23% in C and 19% in N stocks, with significant reductions for herbs+shrubs C stock, MBCS, fine and coarse root C stocks, fine root N stock, NH_4-N and PMNS. Cerrado conversion to both land uses resulted in similar total losses of C and N belowground, which were evident until 50 cm depth, but higher at the 10-30 cm layer.

Key words: aboveground carbon, belowground carbon, belowground nitrogen, carbon stock, Cerrado, *Eucalyptus* plantation, land use change, nitrogen stock, *Urochloa* pasture.

Introduction

The conversion of native vegetation to crop cultures, pastures and silviculture usually results in marked changes on nutrients contents and cycles, and reductions on the carbon and nitrogen stored above and belowground have been frequently reported (Moraes *et al.*, 2002; Guan *et al.*, 2015; Rittl *et al.*, 2017; Sanderman *et al.*, 2018). These stocks depend on the balance among vegetation type and condition, litter input and quality, and organic matter decomposition in the soil (Sotta *et al.*, 2008; Yue *et al.*, 2016), which are highly susceptible to land use/land cover (LULC) changes. Vegetation removal, fertilization procedures and agricultural activities can impair the nutrient cycling (Davidson *et al.*, 2004) and change the organic matter dynamics (Don *et al.*, 2011), what usually cause negative effects on the C and N biogeochemical cycles and storage (Bonini *et al.*, 2018) and result on important ecosystem services drops, especially the nutrient cycling and C sequestration (Brannstrom *et al.*, 2008; Egoh *et al.*, 2009). As a final outcome we may have important feedbacks on climate change (Davidson *et al.*, 2000; Denef *et al.*, 2013), soil fertility and biodiversity. The actual impact of land conversion on nutrient stocks depend on the new activities established and on the original ecosystem features (McGrath *et al.*, 2001).

Estimates of the historical impact of LULC change worldwide state that around 116 million Mg of carbon have already been reallocated from the soil to the atmosphere due to human interference in the natural environments (Sanderman *et al.*, 2018). Due to increased global demand for food, fiber and fuel, the tropics – especially South America and the sub-Saharan Africa – emerged as new agricultural lands, and today are the most intensively converted regions (Gibbs *et al.*, 2010; Phalan *et al.*, 2013). Tropical ecosystems are able to sequester significant amounts of C from the atmosphere, and have great capacity of C storage above and belowground, but high organic matter turnover rates make these ecosystems very sensitive to land use changes (Six *et al.*, 2008). Several studies reported C stock losses in the tropics after the conversion of native vegetation into different land uses (e.g. Don *et al.*, 2011; Deng *et al.*, 2016; Zhang *et al.*, 2017; Fujisaki *et al.*, 2018; Guillaume *et al.*, 2018). Still, soil fertilization and liming, common practices in the tropical regions (Fageria and Baligar, 2008), have resulted in excessive nutrient inputs and adverse effects on water quality and freshwater systems (Matson *et al.*, 1997; Bennet *et al.*, 2001).

In the 1970s Brazil started to emerge as a chief agricultural producer in the tropics. With strong government incentive, Brazil began to invest heavily in modern agriculture for producing commodities, and the Cerrado region was the main target given its relatively low human occupation, flat topography, and soils easily corrected with fertilizers (Ratter *et al.*, 1997; Rada, 2013; Lapola *et al.*, 2014). In the 2000s Brazil became one of the world's greatest producers and exporters of grains, meat and cellulose pulp, mostly coming from the Cerrado (The Economist, 2010; Martinelli *et al.*, 2010). The bad side is that Brazil is now among the 10 leading countries with the highest soil CO_2 emissions for the last few years (Sousa-Neto *et al.*, 2018). According to Santos *et al.* (2019), Brazilian contribution to CO_2 emissions is related to the intensive deforestation, followed mainly by pasture, as the cheapest way to ensure land occupation. The Amazon and Cerrado biomes are responsible for half of the total gas emissions related to LULC changes in the country (Lapola *et al.*, 2014; Sousa-Neto *et al.*, 2018).

On the other hand, the Cerrado biome – that occupies around 2 million km^2 of the Brazilian territory (Ribeiro and Walter, 1998) – is one of the most biodiverse biomes in the planet (Mittermeier *et al.*, 2004) and a biodiversity hotspot, given its high biological value and great threat (Klink and Machado, 2005; Myers *et al.*, 2000). The expansion of agriculture, livestock and forestry since the 1970s resulted in nearly 50% of native Cerrado loss (Lapola *et al.*, 2014), and only 8.3% of the biome is protected as nature reserve (Françoso *et al.*, 2015). Considering the current rate of loss, Cerrado could lose 34% of its natural environment until 2050, leading to extinction more than a thousand endemic species (Straussbug *et al.*, 2017). Besides its importance on storing water, recharging aquifers and feeding the main country's watersheds (Oliveira *et al.*, 2005; Oliveira *et al.*, 2014), Cerrado plays an important part in the global C cycling and nutrients balance (Ribeiro *et al.*, 2011). It has high potential for C storage, mostly belowground (Castro and Kauffman, 1998) but also in the aboveground vegetation and litter (Roquette, 2018). The replacement of the native Cerrado by different land uses also brings great changes in the microbiota, litter quality and nutrient availability, as well as soil compaction (Frazão *et al.*, 2010). In consequence, significant and long-lasting effects to carbon and nutrient pools happen, as they mostly depend on the organic matter decomposition process.

Nearly 30% of the Cerrado biome has been converted to livestock pastures of exotic grasses (Beuchle *et al.*, 2015). Under proper fertilization practices, these pastures are able to store significant amounts of C (Maia *et al.*, 2009; Frazão *et al.*, 2010) and N

(Lilienfein and Wilcke, 2003; Maquere *et al.*, 2008) in the soil, owed to great herbs biomass production; however, the final C balance is usually negative because of tree removal and biomass (carbon) loss (Conant *et al*., 2001). Degraded pastures, on the other hand, revealed significant losses C (eg. Carvalho *et al.* 2010, Salton *et al.* 2011) and N (Guareschi *et al.* 2012, Groppo *et al.* 2015) In the soil. Pastures productivity – which depends on fertilization procedures, soil properties, forage species and microclimatic conditions – seems to be determinant for soil C and N storage capacity in the Cerrado areas (Carvalho *et al*., 2014).

The use of Cerrado lands for the planting of *Eucalyptus* species is another very common practice in the Southeastern part of the Cerrado biome (Durigan *et al.*, 2007), especially in São Paulo state. According to Laclau *et al.* (2010), *Eucalyptus* is the most frequent type of forestry planted in the tropics, but sustainability is pointed as an issue since this culture is commonly established on low-fertile soils, and great nutrient exports happen every 6-7 years of the rotation system, when trees are removed. Studies about the effects on C and N stocks due to the conversion of Cerrado to *Eucalyptus* plantations are scarce and point to ambiguous results; some studies show either no difference, small increments or small reductions of N (Maquere *et al.*, 2008; Pegoraro *et al.*, 2011), while soil C stocks display small increases (Neufeldt, 2002; Maquere, 2004), substantial decreases (Zinn *et al.*, 2002; Montero, 2008) or no change (Lepsch, 1980). Still, studies suggest that *Eucalyptus* plantations in the tropics need increased amounts of fertilizer to counterbalance crop output in the long-term (Laclau *et al.*, 2010). The excessive nutrient input by fertilization and liming procedures in *Eucalyptus* plantations can cause aquatic contamination and high NOx emissions (Verchot *et al.*, 1999; Howarth *et al.*, 2000). Differences in the microclimatic conditions, soil type, vegetation physiognomy, fertilization practices, plantation age, and temporal variations (seasonal and interannual) may explain the lack of consistency found in the limited researches that address the effects of Cerrado conversion to pastures and *Eucalyptus* plantations on C and N stocks.

The quantification of C and N in different storage compartments above and belowground in the converted Cerrado areas will bring new information on nutrients balance and reallocation inside the system, on soil fertility, and C sequestration. Such information will guide the planning and management of Cerrado lands for agricultural uses, carbon sequestration programs, and biodiversity conservation. Therefore, in this study we were guided by the following questions: Is there any effect of LULC on C stocks (above and belowground) and N stocks (belowground) when woodland Cerrado ecosystems are converted to livestock pastures and *Eucalyptus* plantations? If so, what are the gains and losses relative to these stocks? Are C and N stocks reallocated into different compartments after land conversion? Is the overall balance of C and N stocks positive or negative after Cerrado conversion? We thus estimated the belowground C stock – composed of soil organic carbon stock (SOCS) + dissolved organic carbon stock (DOCS) + microbial biomass carbon stock (MBCS) + fine and coarse root C stock – as well as the aboveground C stock – considering litter + herbs/shrubs + tree C stocks –, as well as the overall N stock belowground – composed by total N stock + inorganic N stock (NH_4-N and NO_3–N) + potentially mineralizable nitrogen (PMN) stock + N root (fine and coarse) stock. We assessed stocks from three study landscapes, each one including fragments of Cerrado vegetation and areas converted to livestock *Urochloa* pastures and *Eucalyptus* plantations. Belowground data came from the first 50 cm of soil profile.

Materials and Methods

Study area

Our research was conducted in the Southeastern portion of the Cerrado biome, in São Paulo state, Brazil, at three study sites in Pirassununga and Bauru municipalities (Supplementary Material, Table S1). Climate in the study sites are Koeppen's Cwa in Pirassununga and Aw in Bauru, both warm, humid, with dry winters and wet summers when 75% of the annual rainfall occurs (October to March). Annual rainfall for the region ranges from 1300 mm (Bauru weather station; 22º19' S – 49º02' W; 1945-2001 rainfall series) to 1420 mm (Pirassununga weather station; 21º58' S – 47º28' W; 1954-1999) (DAEE, Department of Water and Electric Energy of São Paulo state). The Cerrado in São Paulo state is currently under intensive anthropic uses, such as agriculture, forestry and livestock pastures, and the native vegetation is limited to several small fragments (Durigan, 2006).

Our research focused on the woodland Cerrado physiognomy, the *cerradão*, which is now dominant in São Paulo state probably due to cattle confinement and prohibition of fire for pasture management (Durigan and Ratter, 2006, 2016). In the absence of fire the open Cerrado physiognomies tend to develop to woody forms (Pivello and Coutinho, 1996). In each of the three study sites – Pirassununga (Pirassununga municipality), Santa Terezinha and Jardim (both in Bauru municipality) (Supplementary Material, Table S1) – we selected one fragment of native *cerradão*, one pasture of

Urochloa decumbens (African grass known as brachiaria), and one *Eucalyptus* plantation (*Eucalyptus urophylla, E. grandis, E. urograndis* or *E. saligna*). These sites were located on flat to gently undulating topography (slope between 0% and 5%), with sandy and dystrophic Red and Dark-Red Latosols (according to Oliveira *et al.*, 1999). The average bulk density from 0-5 and 30-35 cm depth for Cerrado was 1.21 $g.cm^{-3}$, while the target pastures had 1.30 $g.cm^{-3}$ and *Eucalyptus* plantations had 1.27 $g.cm^{-3}$. Soil bulk density values for the two sampled depths (0-5 and 30-35 cm) and study sites are available in Table S2 (Supplementary Material).

To characterize the study sites soils, we determined soil texture by the Bouyoucos densimeter method at 5-10 cm depth (Camargo *et al.*, 1986), and pH in water (EMBRAPA, 1997). We could then attest the similarity of the study sites according to soil features: sand content was over 80% (predominance of coarse sand), pH ranged from 4.95 to 5.93 (Supplementary Material, Table S2). Fertilization procedures in the target pastures and *Eucalyptus* plantations were based on N-P-K application at establishment, in addition to liming in the *Eucalyptus* plantations (see Table S1).

Aboveground C stock

In each fragment of native Cerrado we delimited four 100 m^2 plots to assess tree biomass. We measured tree height and DBH (diameter at breast height) of all trees with DBH ≥ 3 cm. Biomass was determined by the allometric equation proposed by Miguel *et al.* (2017) for the *Cerradão* physiognomy:

$$Y=35,8951 + 1,090*DBH .$$

To determine *Eucalyptus* biomass we applied allometric equations proposed by Silva (1996) for stem, branches and leaves, respectively:

$$Y=442.65*DBH^2$$

$$Y=28.44*DBH^2 – 107.21*H$$

$$Y=35.04*DBH^2 – 134.83*H$$

Where DBH = diameter at breast height and H = total height.

We collected data on DBH, height and tree density for the Santa Terezinha site, and we also used values of similar plantations according to age, species planted and local (Cerrado biome in São Paulo state) and soil type: a 13 year-old *Eucalyptus* plantation in Itatinga municipality (Nicoletti, 2011) and a 5 year-old E. *grandis* plantation in Luiz Antônio municipality (Santana *et al.*, 2008). The C stocks for *Eucalyptus* and native Cerrado trees was determined by multiplying tree biomass by the conversion factor =

0.45, considering that 45% of the total biomass composition is carbon (according to Lieth and Whittaker, 1975; Paiva *et al*., 2011).

To estimate C stocks in herbs+shrubs and litter we established a 140 m transect along which we placed regularly spaced 1 m^2 sampling plots. In seven plots we cut all herbs and the shrubs with DBH <3cm, and collected the litter. Samples were sent to the laboratory for drying and weighting. Herbs+shrubs and litter C stocks were also calculated by multiplying the dry weight by 0.45.

Belowground C and N stocks

In the sampling plots previously established at the 140 m transect in each LULC, we sampled soils at 0-10 and 10-30 cm depth from all sampling points, and at 30-50 cm depth from four sampling points, using an auger of diameter = 17 cm. Soils were sieved at 2 mm and all roots were collected and kept at 4ºC. Two soil sub-samples were separated: a moist-soil sample, kept at 4ºC until analyses, and a dry-soil sample that was air-dried and stored at room temperature prior to analyses. Undisturbed soil samples were collected at 0-5 and 30-35 cm depth (three samples per depth, LULC and site) and dried at 105°C (volumetric ring method; Blake and Hartge, 1986) to calculate soil bulk density. Soil bulk density from 0-5 and 30-35 cm depth was used to estimate C and N stocks for 0-10 and 30-50 cm soil layers respectively; averaged bulk density values from 0-5 and 30-35 cm were used to measure stocks for the 10-30 cm soil layer. Bulk density varied slightly among land uses, being lower values associated to Cerrado followed by pastures and *Eucalyptus* plantations.

Dissolved organic carbon (DOC) and microbial biomass carbon (MBC) were quantified according to Witt *et al.* (2000). SOM content was determined by the Walkley-Black method (Walkley and Black, 1934) in dry soils, and SOC was calculated from SOM results using the correction factor = 1.74 (Nelson and Sommers, 1982). Soil organic carbon stocks (SOCS), dissolved organic carbon stocks (DOCS) and microbial biomass carbon stocks (MBCS) were calculated by the equation (Veldkamp, 1994):
C stock ($Mg.ha^{-1}$) = C variable concentration [$g.kg^{-1}$] * soil bulk density [$g.cm^{-3}$] * soil layer thickness [cm]) /10

Soil ammonium (NH_4–N) and nitrate (NO_3–N) nitrogen were measured by the salicylate/nitroprusside and salicylic acid colorimetric methods, respectively (Cataldo *et al.*, 1975; Baethgen and Alley, 1989). Potentially mineralizable nitrogen (PMN) was determined by soil incubation at 40ºC for 10 days; after incubation, PMN was extracted by 0.625M K_2SO_4, and determination of NH_4–N was made by the salicylate/nitroprusside method. Soil total N content was determined in dry soils by the microkjeldahl method (Raij *et al.*, 1987). Nitrogen stocks (Mg ha^{-1}) were calculated by the equation (Rangel and Silva, 2007):

N stock ($Mg.ha^{-1}$) = (N variable concentration [$g.kg^{-1}$]. soil bulk density [$g.cm^{-3}$]. soil layer thickness [cm]) /10

Root samples were washed with deionized water and classified into fine (<2mm) and coarse roots (≥2mm). Next, they were placed in the oven (60ºC) for 5 days for complete drying, and then weighted in a precision scale. C and N percentages in roots were measured with an atomic absorption spectrometer and averaged for each LULC. Stocks were calculated by multiplying C and N percentage (divided by 100) by root dry weight (Sommer *et al*., 2000).

Statistical Analyses

Data from the different sampling points were averaged for each site and depth (in the case of belowground parameters) so that only a single value per depth or aboveground stock, LULC, and site remained. We used a randomized block design for the belowground C and N stocks (SOCS, DOCS, MBCS, roots C, root N, total N, PMN, NH_4-N, NO_3-N). We analyzed the main effects and interactions of LULC (Cerrado, Pasture or *Eucalyptus* plantation) and soil depth (0-10, 10-30 and 30-50cm), using a mixed-factor analysis of variance, with LULC and depth as fixed factors, and site as random factor. To compare the aboveground biomass (AGB), C stocks above and belowground and the overall stock (belowground + aboveground) for which there is no depth variable, we used one-factor analysis of variance (ANOVA). Within-site differences in C and N stocks between Cerrado and either pasture or *Eucalyptus* plantation were analysed using two-tailed Student's one-sample t-tests. We used the software SPSS 20.0 (for Windows) for statistical analyses.

Results

The mean values of soil P, N and organic matter (SOM) for the studied Cerrados, pastures and *Eucalyptus* plantations up to 200 cm depth show a decreasing trend with depth and higher values in the first 10 cm of the soil profile (Table 1).

Table 1. Soil P, N and soil organic matter (SOM) concentrations for different LULC and five depths (0-10, 10-30, 30-50, 50-100, 100-200 cm). (Means ± Standard Error, n=3).

LULC	Depth (cm)	SOM (%)*	N ($g.kg^{-1}$)*	P ($mg.kg^{-1}$)*
Cerrado	10	1,114 ± 0.149	0,781 ± 0,067	5,735 ± 0,302
	30	0,948 ± 0.110	0,493 ± 0,023	3,806 ± 0,233
	50	0,900 ± 0.080	0,305 ± 0,023	2,843 ± 0,178
	100	0,686 ± 0.059	0,224 ± 0,023	2,097 ± 0,339
	200	0,764 ± 0.258	0,157 ± 0,028	1,656 ± 0,036
Pasture	10	1,082 ± 0.137	0,623 ± 0,192	7,102 ± 1,101
	30	0,925 ± 0.113	0,490 ± 0,138	4,435 ± 0,390
	50	0,815 ± 0.075	0,411 ± 0,121	3,378 ± 0,037
	100	0,688 ± 0.214	0,327 ± 0,103	2,674 ± 0,173
	200	0,473 ± 0.134	0,171 ± 0,010	2,229 ± 0,171
Eucalyptus	10	1,049 ± 0.148	0,704 ± 0,256	9,046 ± 2,098
	30	0,859 ± 0.159	0,545 ± 0,178	5,413 ± 0,737
	50	0,824 ± 0.056	0,385 ± 0,127	5,406 ± 0,545
	100	0,843 ± 0.123	0,267 ± 0,082	3,304 ± 0,373
	200	0,461 ± 0.160	0,212 ± 0,069	2,639 ± 0,524

*Results were averaged from the seven sampling points established in each site (Pirassununga, Sta. Terezinha and Jardim) for the 0-10 and 10-30 cm depth, and from four sampling points for the 30-50, 50-100 and 100-200 cm depth, all collected in the dry and wet seasons.

The overall C stock (above + belowground) for Cerrado was 90.89 $Mg.ha^{-1}$, from which 45% were stored in the aboveground biomass (AGB: litter, herbs+shrubs, trees) and 55% in the first 50 cm of soil; pastures showed 53% decrease on the overall C stock (42.99 $Mg.ha^{-1}$) compared to the native vegetation, being 93% stored in the soil (40.12 $Mg.ha^{-1}$) and only 7% (2.87 $Mg.ha^{-1}$) is in the AGB; *Eucalyptus* plantations showed 20% increase in the overall C stock compared to Cerrado (109.35 $Mg.ha^{-1}$), from which 35% is belowground (38.43 $Mg.ha^{-1}$) and 65% (70.92 $Mg.ha^{-1}$) is in the AGB (Figure 1). There was a significant LULC effect on the overall C stock (Table 1).

Aboveground C stocks decreased 94% in pastures and increased 80% in *Eucalyptus* plantations compared to Cerrado vegetation, with significant LULC effect (Table 1). C stocks of litter, herbs+shrubs and trees also had a significant effect from LULC (Table 1). Litter C socks were similar between Cerrado and *Eucalyptus* plantations (respectively 4.68 $Mg.ha^{-1}$ and 5.16 $Mg.ha^{-1}$) but much lower in pastures (1.13 $Mg.ha^{-1}$), while herbs+shrubs C stock was higher in pastures (1.74 $Mg.ha^{-1}$) and much lower in *Eucalyptus* plantations (0.04 $Mg.ha^{-1}$) compared to Cerrado (0.39 $Mg.ha^{-}$

^{1}) (Figure 1). The highest contrast was between tree C stock in *Eucalyptus* plantations (65.73 Mg.ha^{-1}) and pastures (nil) (Figure 1). Belowground C stocks (50 cm depth) decreased for both land uses relative to native Cerrado (Figure 1), but there was no significant LULC effect (Table 1). Still, LULC effect was significant for root C stock (fine + coarse roots) and MBCS (Table 2). Root C in pastures and *Eucalyptus* plantations were much lower than in Cerrado (respectively 6.56 Mg.ha^{-1}, 5.77 Mg.ha^{-1} and 15.75 Mg.ha^{-1}) (Figure 1). The labile C stock (DOCS + MBCS) was higher in Cerrado (1.03 Mg.ha^{-1}), followed by pastures (0.90 Mg.ha^{-1}) and *Eucalyptus* (0.89 Mg.ha^{-1}).

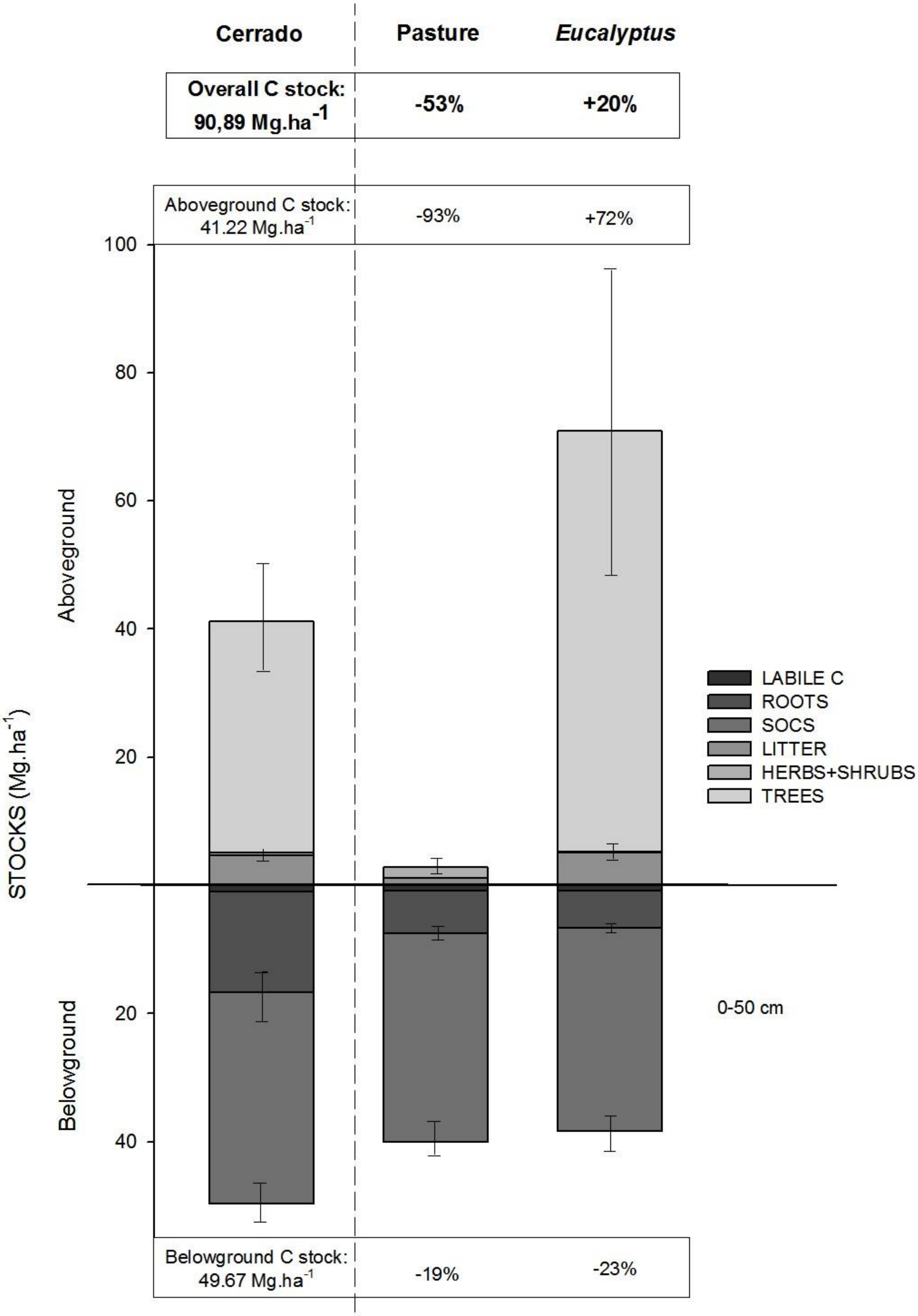

Figure 1. Above and belowground C stocks (Mg.ha^{-1}) – tree C stock, herbs+shrubs C stock, litter C stock, SOCS, Root C stock (fine + coarse root), labile C stock (DOCS + MBCS) – for the studied LULC: native Cerrado fragments, pastures and *Eucalyptus* plantations. (Means ± Standard error, n=3)

Table 2. LULC effect on C stocks of litter, herbs+shrubs, and trees, belowground C (Soil organic carbon stock (SOCS) + dissolved organic carbon stock (DOCS) + microbial biomass carbon stock (MBCS) + total root C stock), aboveground C stock (litter C + herbs+shrubs C + trees C stock) and overall C stock (aboveground + belowground C stock). Results of one-factor ANOVA (F and p values). In bold: significant effects; in bold and italics: marginally significant (p≤0.1) effect.

Effect	**LITTER**	**HERBS/ SHRUBS**	**TREES**	**BELOW**	**ABOVE**	**OVERALL**
LULC	F=14.57 **p=0.005**	F=19.0 **p= 0.003**	F=1.8 **p=0.029**	F=3.2 p=0.111	F=7.7 **p=0.022**	F=6.7 **p=0.029**

Table 3. Effects of land use or cover (LULC), depth (0-10, 10-30 and 30-50 cm), site (Pirassununga, Santa Terezinha and Bauru) and their interactions on stocks of soil organic carbon (SOCS), dissolved organic carbon (DOCS), microbial biomass carbon (MBCS), fine root, coarse root and total root (fine + coarse). Mixed- factor Analysis of Variance; F statistic and p value for main effects and interactions. In bold: significant effects; in bold and italics: marginally significant (p≤0.1) effects.

Effect	**SOCS**	**DOCS**	**MBCS**	**FINE ROOT**	**COARSE ROOT**	**TOTAL ROOT**
LULC	F=0.4 p=0.964	F=0.1 p= 0.858	F=11.7 **p=0.021**	F=3.1 p=0.155	F=8.0 **p=0.040**	F=9.1 **p=0.033**
Depth (D)	F=8.8 **p=0.001**	F=24.5 **p=0.005**	F=15.0 **p=0.013**	F=17.4 **p=0.011**	F=1.8 p=0.274	F=4.1 p=0.107
Site (St)	F=8.8 **p=0.004**	F=0.5 p=0.627	F=0.5 p=0.598	F=1.6 p=0.310	F=0.8 p=0.538	F=1.0 p=0.483
LULC x D	F=0.2 p=0.912	F=0.8 p=0.552	F=0.2 p=0.939	F=1.5 p=0.294	F=2.4 p=0.136	F=2.3 p=0.142
LULC x St	F=1.9 p=0.159	F=0.7 p=0.635	F=0.7 p=0.594	F=1.7 p=0.235	F=1.1 p=0.414	F=0.9 p=0.464
D x St	F=0.2 p=0.930	F=O.6 p=0.693	F=0.8 p=0.577	F=1.7 p=0.240	F=1.6 p=0.249	F=1.4 p=0.304

Changes in belowground C stocks caused by the conversion of Cerrado to pastures and *Eucalyptus* plantations showed mostly losses, especially in the 10-30 cm depth (Figure 2). The MBCS had significant decreases throughout the soil profile in both pastures (-0.13 $Mg.ha^{-1}$, 44% decrease for 0-50cm depth) and *Eucalyptus* plantations (-0.10 $Mg.ha^{-1}$, 34% decrease for 0-50cm depth). Fine root C stock had significant losses for *Eucalyptus* plantations, especially in the 10-30 cm depth (-0.65 $Mg.ha^{-1}$, 24% decrease) while coarse root C significantly reduced in both converted lands, also at 10-30 cm depth, with 90% decrease for pastures (-5.76 $Mg.ha^{-1}$) and 92% decrease for *Eucalyptus* (-5.91 $Mg.ha^{-1}$) (Figure 2). Changes in SOCS and DOCS were not significant.

Gains and losses in AGB carbon stocks caused by the conversion of Cerrado to pastures revealed decreases in litter C stock (-3.55 $Mg.ha^{-1}$, 76%), a fourfold increase in herbs and shrubs C stock (+1.35 $Mg.ha^{-1}$), besides the absence of trees (Figure 3). *Eucalyptus* plantations, on the other hand, showed losses in herbs+shrubs C stock (-0.35

Mg.ha^{-1}, 90%), gain on litter stock (-0.48 Mg.ha^{-1}, 10%) and increase in tree C stock (+29.58 Mg.ha^{-1}, 82% increase) (Figure 3).

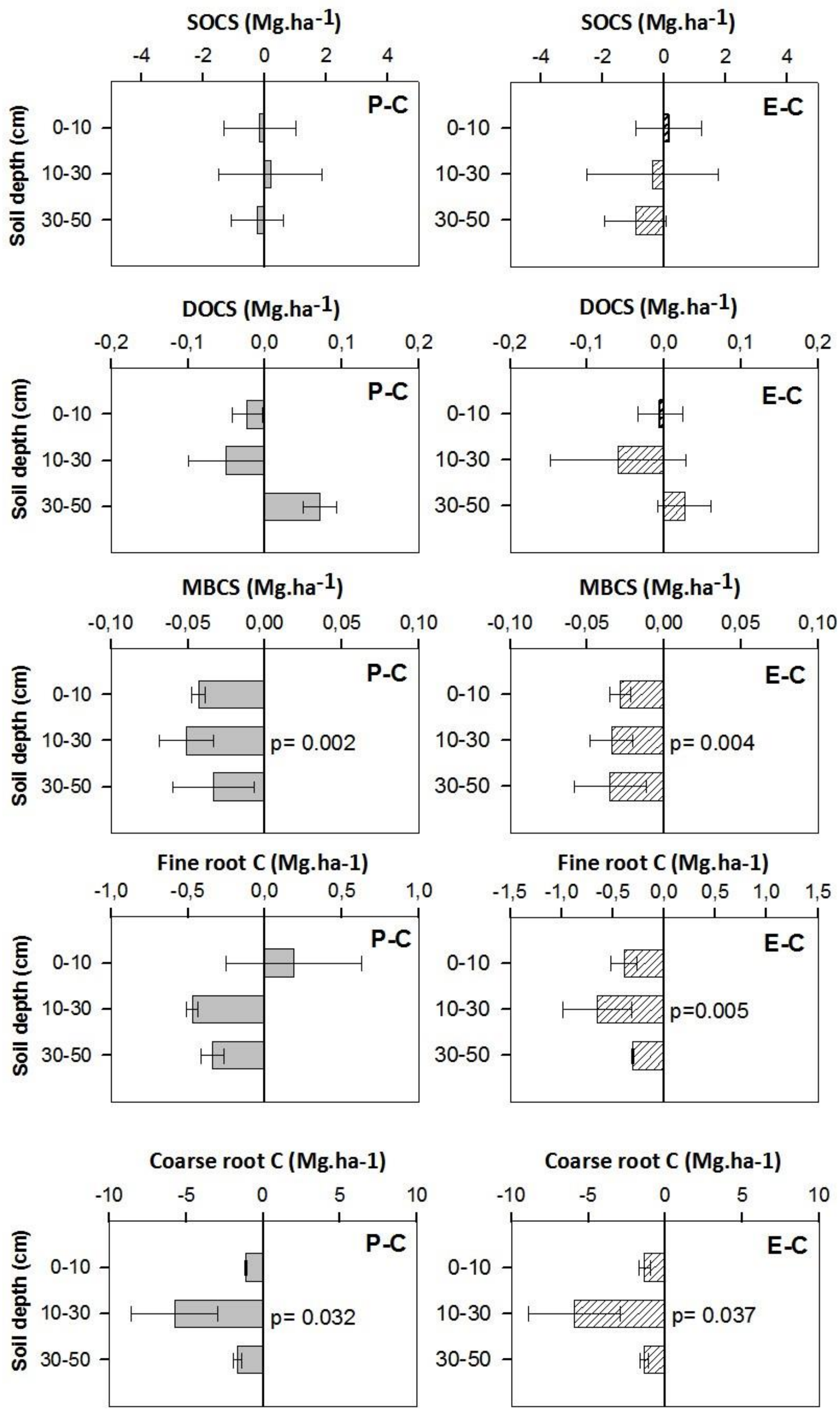

Figure 2. Average changes of soil organic carbon stock (SOCS), dissolved organic carbon stock (DOCS), microbial biomass carbon stock (MBCS), fine and coarse root C stocks, at 0-10, 10-30, and 30-50 cm depth due the conversion of Cerrado into pastures (P-C) and *Eucalyptus* plantations (E-C). Negative values represent net losses and positive values represent net gains. (Mean ± standard error; n = 3)

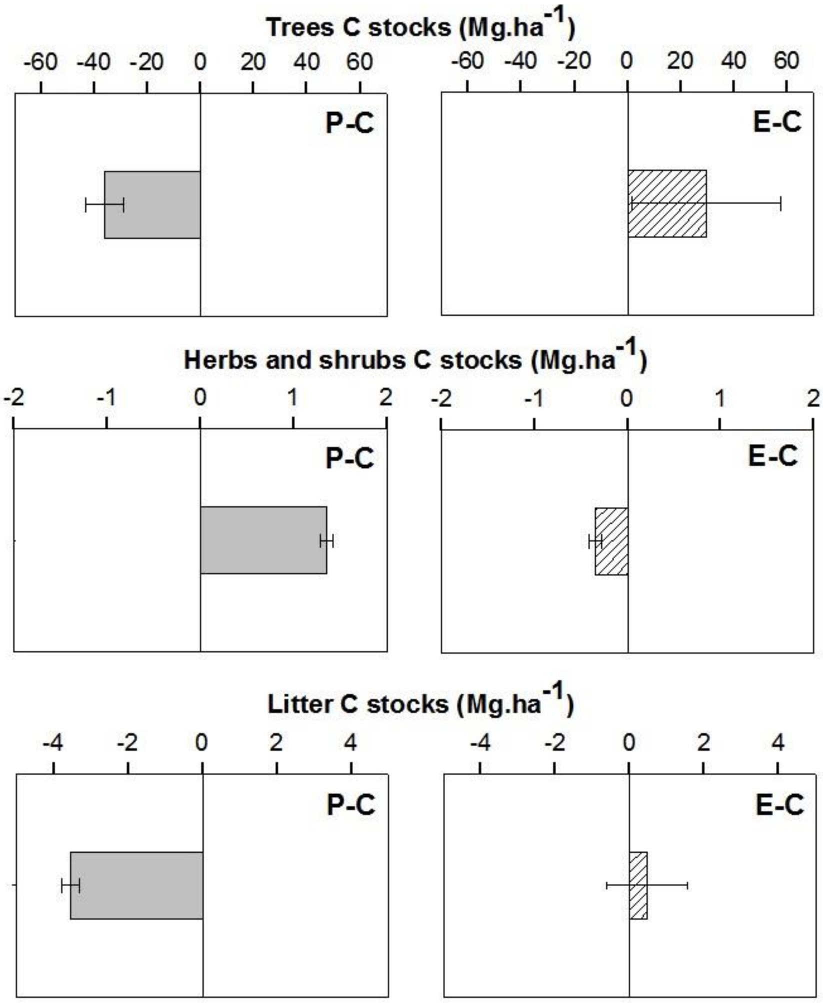

Figure 3. Average changes of aboveground C stocks – trees, herbs+shrubs and litter C stocks – due the conversion of Cerrado into pastures (P-C) and *Eucalyptus* plantations (E-C). Negative values represent net losses and positive values represent net gains. (Mean ± standard error; n = 3)

The overall belowground N stocks (potentially mineralizable nitrogen stock [PMNS] + inorganic N stock [NH_4-N and NO_3-N] + root N stock [fine root + coarse root]) revealed 25% decrease in pastures and 19% in *Eucalyptus* plantations compared to Cerrado (Figure 4), but no significant LULC effect (F=1.1; p=0.386). Nevertheless, when considered separately, all N stocks compartments had significant LULC effect, except for NH_4-N (Table 3). In both converted land uses, PMNS and root N stocks showed consistent decreases compared to Cerrado. Total root N followed the order Cerrado>Pasture>*Eucalyptus* and PMNS followed the order Cerrado>*Eucalyptus*>Pasture (Figure 4). Pastures also had NO_3-N decrease, while *Eucalyptus* plantations had higher NO_3-N and total N stocks than Cerrado (Figure 4). Total N, PMNS, NO3-N and fine root N stock had significant Depth and Site effect (Table 3).

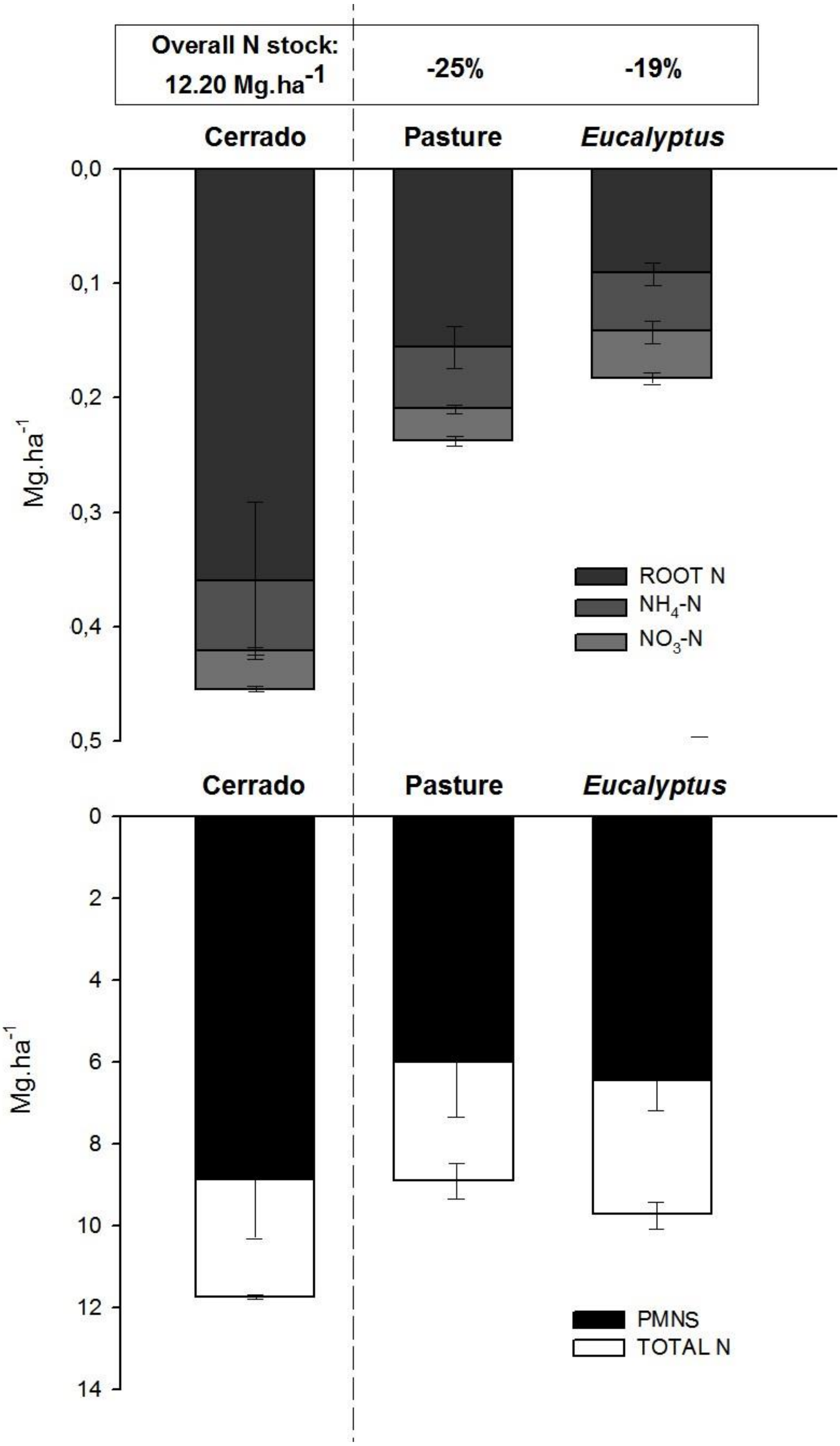

Figure 4. Belowground N stocks: overall N stock, potentially mineralizable nitrogen stock (PMNS), inorganic forms NH4-N and NO3-N stocks, root N stock (fine root + coarse root) – and overall belowground N stock (sum of all N stocks: total N stock + PMNS + inorganic N + total root N), for Cerrado, pastures and *Eucalyptus* plantations. (Means ± Standard error, n=3)

Table 4. Effects of land use or cover (LULC), depth (0-10, 10-30 and 30-50 cm), site (Pirassununga, Santa Terezinha and Bauru) and their interactions on stocks of total N, potentially mineralizable nitrogen (PMN), NH4-N, NO_3-N, fine root N, coarse root N and total root N (fine + coarse). Mixed- factor Analysis of Variance; F statistic and p value for main effects and interactions. In bold: significant effects; in bold and italics: marginally significant ($p \leq 0.1$) effects.

Effect	**TOTAL N**	**PMNS**	**NH_4-N**	**NO_3-N**	**FINE ROOT**	**COARSE ROOT**	**TOTAL ROOT**
LULC	F=29.8 **p=0.003**	F=4.6 ***p= 0.090***	F=3.1 p=0.154	F=29.8 **p=0.003**	F=19.6 **p=0.008**	F=9.5 **p=0.030**	F=12.6 **p=0.018**
Depth (D)	F=16.5 **p=0.011**	F=15.5 **p=0.013**	F=3.5 p=0.133	F=16.5 **p=0.011**	F=19.7 **p=0.008**	F=1.8 p=0.263	F=4.2 p=0.105
Site (St)	F=19.0 **P<0.001**	F=34.2 **P<0.001**	F=0.7 p=0.500	F=19.0 **p<0.001**	F=5.5 **p=0.031**	F=1.4 p=0.297	F=1.4 p=0.290
LULC x D	F=3.8 **p=0.050**	F=1.9 p=0.209	F=0.4 p=0.827	F=3.8 ***p=0.051***	F=2.2 p=0.150	F=2.5 p=0.119	F=2.7 p=0.110
LULC x St	F=0.6 p=0.670	F=7.3 **p=0.008**	F=1.4 p=0.322	F=0.6 p=0.670	F=1.5 p=0.302	F=1.1 p=0.423	F=0.9 p=0.466
D x St	F=1.7 p=0.228	F=1.7 p=0.240	F=0.7 p=0.612	F=1.8 p=0.223	F=2.1 p=0.165	F=1.7 p=0.239	F=1.6 p=0.269

The average gains and losses in belowground N stocks related to the conversion of Cerrado to pastures and *Eucalyptus* plantations showed losses in the uppermost soil layers, except for PMN, fine and coarse root N stocks that revealed losses in all layers. In pastures, decreases in NO_3-N stock at 0-10 cm were of 42%, and decreases in PMNS rates at 0-10 and 10-30 cm were 36% and 32%, respectively (Figure 5). *Eucalyptus* plantations showed a 20% reduction in NH_4-N stock at 10-30 cm and 46% decrease in PMNS at 0-10 cm depth (Figure 5). Fine root N stocks had significant losses (24%) at 10-30 cm depth for *Eucalyptus* plantations. Coarse root N stocks also showed significant losses at 10-30 cm depth but for both agricultural uses, where pastures had 89% less than Cerrado, whereas *Eucalyptus* plantations showed reduction of 91% (Figure 5). Less pronounced changes were observed below 30 cm soil depth.

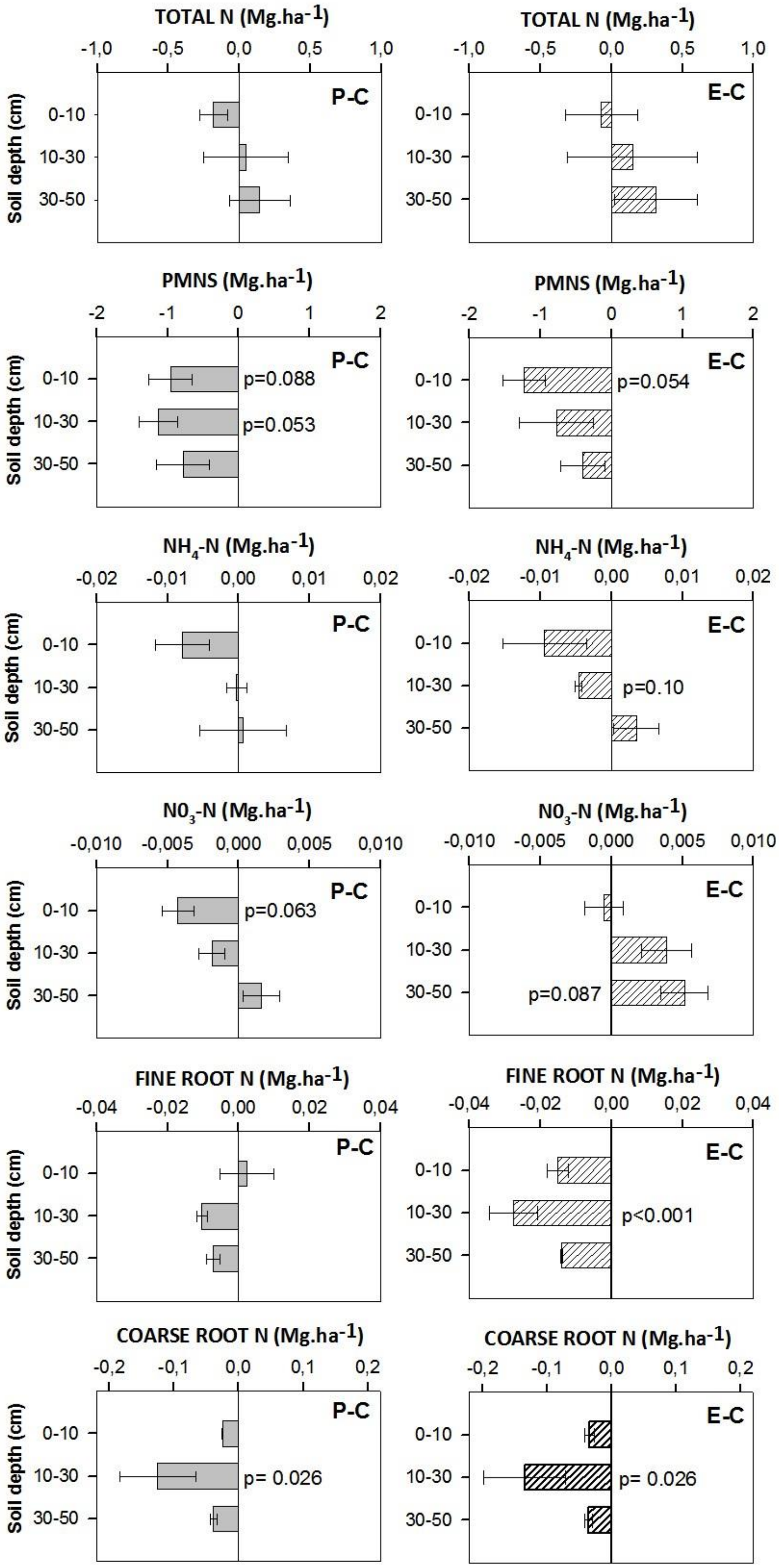

Figure 5. Average changes for the NH_4-N and NO_3- N stocks, total N stock and PMNS at 0-10, 10-30, and 30-50 cm depth in the soil due the conversion of Cerrado into pastures (P-C) and *Eucalyptus* plantations (E-C). Negative values represent net losses and positive values represent net gains (Mean ± standard error; n = 3). Marginally significant P values indicated.

Discussion

The replacement of native Cerrado (*cerradão* physiognomy) by pastures and *Eucalyptus* plantations caused a redistribution of carbon (above and belowground) and nitrogen (belowground) in the system. Relative to carbon, Cerrado showed a more balanced C distribution aboveground (45%) and belowground (up to 50 cm down – 55%). In pastures, C is mostly stored belowground, as the absence of trees and the little amount of litter maintain low AGB and a negative final C-balance (above + below) compared to Cerrado, as the overall C stock is reduced by half. On the other hand, *Eucalyptus* plantations store C mostly in the AGB and show a positive final C-balance and an increment in overall C stock due to huge tree biomass.

Belowground, the overall C and N stocks decreased for both types of Cerrado conversion. *Eucalyptus* plantations point to a critical situation, in which *Eucalyptus* trees - which have large nutrients uptake – reallocate C and N from the soil to the AGB, which is then removed by tree harvesting every seven years (in the case of commercial plantations). According to Barreto *et al*. (2012), the cutting of *Eucalyptus* trees by the age of five years removes 34% of the AGB nitrogen. *Eucalyptus* foliage, bark and litter contain high amounts of nutrients and almost 50% of the plant N, evidencing the need of management procedures that retain most of the nutrients in the place. Soil exposure after tree harvesting and the agricultural practices for a new production cycle cause lixiviation and nutrient loss, which also contribute to C and N cycling disruption and storage reduction (White *et al*., 1993; Barreto *et al*., 2012). For pastures, the establishment of monospecific grass plantations promotes losses on litter C stocks. Aboveground, the increment of herbs C do not compensate at all the removal of native trees and litter quality (based on a single exotic species) influences the microbial community that may explain MBCS massive reduction and losses of inorganic forms of N (NH_4-N and NO_3-N) and PMNS, which are directly related to the microbial activity. Microbial processes, such as mineralization and immobilization, are essential regulators of the N availability (Vargas and Scholles, 1998). Microclimate change due to the absence of trees, soil compactation by cattle trampling, and changes in litter quantity and quality in pastures have been reported to alter the microbial community that consequently changes the organic matter decomposition and affects the nutrient cycling (Batlle-Bayer *et al*., 2010; Oliveira *et al*. 2016; Vinhal-Freitas *et al*. 2017).

Morais *et al*. (2013) found litter C stock of 5 Mg.ha^{-1} in the Cerrado, a value similar to ours. In *Eucalyptus* plantations litter C stock was higher than in the Cerrado as a direct consequence of higher tree biomass production. Similarly to us, Montero (2008) also found higher litter biomass for *Eucalyptus* plantations (11.71 Mg.ha^{-1}) compared to native *cerradão* (9.18 Mg.ha^{-1}). Ribeiro *et al*. (2018) explain the higher litter biomass and litter C stocks in *Eucalyptus* plantations compared to native Cerrado (*cerradão* physiognomy) due to greater deposition and low decomposition rates of the plant material, as litter decomposition showed to be faster in the native Cerrado than in areas converted to *Eucalyptus* plantations. In our research, litter increase did not result on labile C gains; MBCS was substantially reduced, as the PMN, and the lowest NH_4-N stocks were found in *Eucalyptus* plantations, what indicates poor litter quality. According to Pegoraro (2007), high quantities of more coarse and lignified plant residues are found in *Eucalyptus* stands, while pasture litter has less lignin and tannin and is easier to decompose by microbes (Mendham *et al*., 2004). Besides, *Eucalyptus* leaves have antibacterial substances that hinder organic matter decomposition (FIorenzano, 1957; Bruna *et al*., 1989), as well as high C:N ratio (Pillon *et al*. 2011), that reduces bacteria populations (including nitrifying bacteria) (Ferreira *et al*., 2016).

Eucalyptus plantations showed higher levels of NO_3-N compared to Cerrado, which can be associated to soil fertilization practices. Contrarily to our results, Gama-Rodrigues *et al*. (2008) and Ribeiro *et al*. (2011) found higher NO_3-N concentrations in the native vegetation of Cerrado and Atlantic Forest than in *Eucalyptus* plantations, although these studies do not calculate stocks. The Cerrado conversion to pastures resulted in lower DOCS, especially at 10-30 cm depth, where C and N stocks of fine and coarse roots were also reduced. DOC comes from root exudates, plant residues and decomposing litter (Chantigny, 2003); it is part of the most labile SOC pool (Strosser, 2010) and represents an essential part of the global C cycle (Siemens, 2003), being also an important C source for the microbial community (Wang *et al*., 2003). *Eucalyptus* plantations showed higher DOCS reduction than pastures at 0-30 cm depth compared to Cerrado, probably related to litter quality and massive drops on C and N roots stocks.

In pastures, fine root C and N stocks increase at 0-10 cm depth and decrease at 10-30 and 30-50 cm, while in *Eucalyptus* plantations these stocks decrease through all depths. The root system in pastures is expected to be well-developed up to 100 cm depth, but the first 10 cm concentrate almost half of the root biomass (Teixeira and Bastos, 1989; Rangel and Silva, 2007). Still, *Eucalyptus* roots C is concentrated mainly at the first 30 cm of the soil profile (Alcântara *et al*., 2004). Likewise, the conversion of Cerrado to pasture and *Eucalyptus* resulted in great losses of coarse root C and N, especially at 10-30 cm depth. Rodin (2004) reported losses of coarse root biomass of about 70-87% in Cerrado converted to *Urochloa* pastures, a result similar to ours. Abdala *et al*. (1998), Aduan *et al*. (2003) and Morais *et al*. (2013) found most root biomass in native Cerrado at the first 30 cm of the soil profile, which can explain higher root losses at the uppermost soil layers with land use conversion.

The SOCS values found in our study are on a smaller scale compared to other authors' values for the Cerrado converted to pastures (Marchão *et al*., 2009; Wantzen *et al*., 2012) or to *Eucalyptus* plantations (Corazza *et al*., 1999). Still, differently of what we found, Corazza *et al*. (1999) reported higher SOCS at 0-20 cm depth for *Eucalyptus* plantations (44.87 $Mg.ha^{-1}$) and pastures (42.18 $Mg.ha^{-1}$) related to native Cerrado (39,77 $Mg.ha^{-1}$).

According to Pegoraro (2007), tropical soils cultivated with *Eucalyptus* have higher potential for C storage compared to low productive pastures. However, our results indicate similar C losses for *Eucalyptus* plantations and pastures in the Cerrado soils, and also similar reductions for N stocks in both land uses. The fast production of huge biomass by *Eucalyptus* trees and its harvesting on a short rotation system are known to deplete soil nutrients (White *et al*., 1993). In the tropics, *Eucalyptus* plantations have shown increasing fertilization requirements in the long-term due to nutrient uptake and losses (Laclau *et al.*, 2010). The conversion of Cerrado to poorly managed pastures (a common practice in Brazil) usually decreases SOCS over time (e.g. Salton, 2005; Maia *et al*., 2009; Carvalho *et al*., 2010; Salton *et al*., 2011), with greater losses reported for coarse textured soils (Silva *et al*., 1994; Dieckow *et al*., 2009).

Our findings indicate that there is a tendency to loss of certain forms of C and N stocks – especially C – not only in the most superficial soil layer but along the soil profile (50 cm), and highlight the need for better planning on land use and agronomic practices adopted.

Conclusion

The conversion of woodland Cerrado to pastures and *Eucalyptus* plantations resulted in C (above and belowground) and N (belowground) losses, and also in redistribution among different compartments. Pastures had an overall negative balance in total C stock compared to the native vegetation, showing significant losses aboveground (tree and litter C stocks) despite herbs C stock increment, and also significant C and N losses belowground. *Eucalyptus* plantations, on the other hand, showed a positive balance of overall C stock due to the AGB (tree and litter C stocks). The harvesting of *Eucalyptus* trees on a 6-7 year cycle (usually adopted in commercial plantations) results in high nutrient exports and cycling disruption.

Both land conversions had similar belowground C and N losses, especially for MBCS, PMNS, NH_4-N and coarse root C and N stocks compartments. Although the losses of C and N observed more intensely at 10-30 cm depth, they tended to continue down to 50 cm depth and evidenced that effects of the Cerrado conversion to agricultural uses on soil chemical properties may exceed the uppermost soil layer.

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Supplementary Material

Table S1. Location, age at sampling time, previous land use/cover (LULC), and fertilization of each target LULC at the study sites.

Site name/ Municipality	Land use / cover	Coordinates	Age	Previous land use/ cover	Fertilization
Pirassununga/ Pirassununga	Cerradão	21°56'35" S; 47°28'26" W	original	Cerrado	None
	Pasture - *Urochloa decumbens*	21°56'42" S; 47°28'11" W	> 20 years	Corn and soybean	N-P-K 20-20-20 + Zn, Cu, at establishment
	Silviculture *Eucalyptus saligna/ E. Urophylla*	21°58'10" S; 47°28'25" W	16 years	Pasture	N-P-K 6-30-6 at establishment
Santa Terezinha/ Bauru	Cerradão	22°18’50" S; 49°00'13" W	original	Cerrado	None
	Pasture *U. decumbens*	22°18'52" S; 48°58'29" W	13 years	Cerrado	N-P-K yearly
	Silviculture *E. urophylla/ E. grandis/ E. urograndis*	22°19'35" S; 48°58'21" W	12 years	Cerrado	N-P-K 6-30-6 at establishment and on January 2011
Jardim/Bauru	Cerradão	22°20'45" S; 49°00'48" W	original	Cerrado	None
	Pasture *U. decumbens*	22°21’45" S; 49°01'34' W	> 20 years	Fruit (mango)	None
	Silviculture *E. urophylla*	22°13’38" S; 49°12' 01" W	8 years	Pasture	N-P-K 13-13-13 at establishment; N-P-K 20-5-20 twice after planting

Table S2. Soil particle size distribution (Bouyoucos densimeter method; Camargo *et al*. 1986), pH (mean values; n=3) at the 0-10 cm depth and soil bulk density (mean values; n=3) from 0-5 and 30-35 cm depth for the study sites and target land uses /cover (LULC).

Site	**LULC**	**Coarse Sand (%)**	**Fine Sand (%)**	**Total sand (%)**	**pH**	**Depth* (cm)**	**Bulk Density ($g.cm^{-3}$)**
Pirassununga	Cerrado	51.1	43.7	94.7	5.19	0-5	1.13
						30-35	1.26
	Pasture	51.0	43.0	94.0	5.93	0-5	1.06
						30-35	1.38
	Eucalyptus	42.1	40.0	82.1	5.86	0-50	1.14
						30-35	1.11
Sta. Terezinha	Cerrado	53.4	39.7	93.1	5.13	0-5	1.29
						30-35	1.37
	Pasture	58.0	33.0	91.0	5.52	0-50	1.31
						30-35	1.37
	Eucalyptus	50.5	37.3	87.8	4.95	0-5	1.36
						30-35	1.30
Jardim	Cerrado	48.2	36.7	85.0	5.38	0-5	1.02
						30-35	1.20
	Pasture	51.8	34.4	86.2	5.75	0-5	1.18
						30-35	1.30
	Eucalyptus	46.3	35.2	81.5	5.60	0-5	1.42
						30-35	1.44

*Sampled depths for bulk density.

GENERAL CONCLUSION

The conversion of native Cerrado to brachiaria pastures and *Eucalyptus* plantations resulted in significant alterations in the stocks of carbon and nitrogen. Storage capacity in pastures was reduced as they had an overall loss in C and N stocks above and belowground. On the other hand, storage capacity in *Eucalyptus* plantations was reduced essentially belowground. The labile fraction of SOC and soil N pools responded to LULC changes with significant losses in different compartments. Pastures and *Eucalyptus* plantations had a less efficient microbial community and showed lower potential for mineralization. The losses in the most labile fractions of soil C and N after conversion point to larger changes in more stable pools in the long-term, which can lead to storage capacity decreases, lower soil fertility and increased gas emissions to the atmosphere.

Seasonality influenced soil functioning and affected some of the C and N variables assessed in chapters 1 and 2. The LULC impact was stronger in the dry season for SOC labile pools and NH_4-N. *Cerradão* had higher microbial activity in the dry season (due to higher DOC rates at these sites), while pastures and *Eucalyptus* plantations results pointed to a less efficient microbial community regarding litter decomposition (higher qCO_2 rates). Our results highlight the importance of considering seasonality on analyzing the effects of land use conversion in Cerrado areas and also for management procedures, as the two pronounced climatic seasons of the biome create two contrasting stresses that will affect, especially, the most labile fraction of the soil organic matter.

The native vegetation substitution by pasture or silviculture substantially change the environment features: exotic species provide litter with characteristics very different than those of the original vegetation, fertilization and other agricultural procedures – especially in the *Eucalyptus* plantations – or the lack of management practices – in pastures – might explain the impacts on C and N variables. The new environmental features showed to be detrimental to microbial growth and activity, as their capacity to convert C into microbial biomass decreased and resulted in SOC instability in both land uses.

Carbon and nitrogen stock losses where higher at 10-30 cm depth, but reached the 50 cm in some cases, evidencing that effects of land use conversion in the Cerrado biome can surpass the uppermost soil layer. Deeper soils showed significant importance in C and N cycles (up to 50 cm depth) in the Cerrado biome, and should be included in future studies with land use impact in these areas. Carbon variables in pastures and *Eucalyptus* plantations had a different response to depth: MBC and DOC showed a "reversed" pattern with lower values at 0-10 cm and higher values at 10-30 cm layer, opposed to the native Cerrado, which had a clear decrease along soil depth. These "reversed" pattern can be related to soil fertilization and management practices, which could be reallocating the most fertile level to deeper layers.

The results presented here can contribute to further land use planning that might considerer C and N storage capacity in the system. We encourage more studies approaching the impact of land conversion on carbon and nitrogen biogeochemical cycles in the Cerrado biome, as they are still very scarce.