

**University of São Paulo
“Luiz de Queiroz” College of Agriculture**

**Soil engineering by macroinvertebrates: controls on soil organic matter
storage across land use change**

André Luiz Custódio Franco

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

**Piracicaba
2015**

André Luiz Custódio Franco
Agronomist

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Advisor:
Prof. Dr. **CARLOS CLEMENTE CERRI**

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RESUMO

Macroinvertebrados e os processos de engenharia do solo: controles sobre a estocagem de matéria orgânica do solo em áreas com mudanças no uso da terra

Globalmente mudanças no uso da terra (MUT) com o aumento da intensidade de uso do solo têm levado a uma diminuição da matéria orgânica do solo (MOS). A redução do estoque de C do solo após MUT tem sido acompanhada por uma desestabilização da estrutura do solo e aumento da susceptibilidade a erosão. A desestabilização da estrutura também é concomitante com a perda da biodiversidade do solo e, em particular, da comunidade de macroinvertebrados do solo. O foco deste trabalho é o efeito de MUT com aumento na intensidade de uso do solo sobre a macrofauna do solo, agregação e alocação da MOS. Nossa hipótese é que MUT reduz a diversidade da macrofauna do solo e, consequentemente, diminui os processos de engenharia de solo, resultando na desestabilização da estrutura do solo e prejudicando a capacidade do solo para proteger fisicamente a MOS da decomposição dentro de agregados estáveis, finalmente levando a redução dos estoques de C após MUT. Foi realizada uma pesquisa em 3 cronomsequências de uso da terra que compreendem vegetação nativa (NV), pastagem (PA), e cana-de-açúcar (CA) na região Centro-Sul do Brasil. Esta MUT fornece um gradiente de intensidade de uso do solo e é projetada para adicionar 6,4 Mha de novas áreas de CA no Brasil até 2021. Em cada ponto de amostragem de solo blocos de 25 x 25 cm e 5 x 5 cm a 10 cm de profundidade foram coletados simultaneamente das camadas 0-10 cm, 10-20 cm e 20-30 cm de solo, para isolamento da macrofauna e fracionamento de agregados, respectivamente. Foi observada uma redução média de 89% na densidade da comunidade da macrofauna quando CA substitui PA, e uma perda de 39% da diversidade de grupos. Nossos resultados mostraram que, em um intervalo de texturas do solo (16-66% de argila), tal perda de biodiversidade foi fortemente correlacionada com a desestabilização da estrutura do solo após MUT. Estas observações indicam consistentemente que a abundância de animais detritívoros, especialmente minhocas e cupins, pode ser um preditor significativo de transformações da estrutura do solo em MUT. Além disso, a forte redução na abundância de minhocas foi fortemente e positivamente correlacionada com a diminuição do C alocado intra macroagregados. Como resultado, após mais de 20 anos de cultura de CA houve perdas de 40 e 35% dos estoques de C e N, respectivamente, resultando em uma taxa de emissão de C de $1,3 \text{ Mg ha}^{-1} \text{ ano}^{-1}$. Esta perda de C ocorreu principalmente no C associado aos macroagregados, como um resultado da reciclagem mais rápida dos macroagregados sob CA. Em resumo, os resultados aqui apresentados fornecem uma explicação mecanicista a respeito de porque há esgotamento do C do solo quando aumenta-se a intensidade de uso do solo em ambientes tropicais: a enorme redução na abundância de invertebrados “engenheiros do solo” após MUT prejudica a capacidade do solo para proteger fisicamente a MOS da decomposição dentro de agregados estáveis, e, portanto, é um mecanismo primário controlando a redução dos estoques de C no solo relacionada a MUT.

Palavras-chave: Agregação; Biodiversidade do solo; Estoques de carbono; Estrutura do solo; Expansão da cana-de-açúcar; Funcionamento de ecossistemas; Macrofauna

ABSTRACT

Soil engineering by macroinvertebrates: controls on soil organic matter storage across land use change

Globally land use change (LUC) with increasing in land use intensity has led to a decrease in soil organic matter (SOM). The reduction of soil C stock across LUC has been accompanied by a destabilization of soil structure and increases the soil erosion susceptibility. The destabilized soil structure is also concomitant with a loss of soil biodiversity and in particular, soil macroinvertebrate community. The focus of this dissertation is the effect of LUC with increasing in land use intensity on soil macrofauna, aggregation and SOM allocation. We hypothesized that LUC reduces soil macrofaunal biodiversity and consequently decreases soil engineering processes, resulting in destabilization of soil structure and impairing the ability of soil to physically protect SOM from decomposition inside stable aggregates, finally leading to depleted SOC stocks across LUC. In order to test these hypotheses, we conducted a field survey in 3 chronosequences of land use comprising native vegetation (NV), pasture (PA), and sugarcane crop (SC) in Central-Southern Brazil. This land use sequence provides a gradient of land use intensity and is projected to add 6.4 Mha of new sugarcane areas in Brazil by 2021. At each sampling point soil blocks of 25 X 25 cm and 5 x 5 cm to 10 cm depth were simultaneously collected from 0-10 cm, 10-20 cm, and 20-30 cm soil layers, for macrofauna isolation and aggregate fractionation, respectively. Within a radius of 6 meters around each sampling point, 12 subsamples were also collected from the same soil layers, and combined for total soil C and N concentration. An average reduction of 89% in the density of the soil macrofaunal community was observed when SC replaces PA, and a loss of 39% in the diversity of macrofauna groups. Our findings showed that, over a range of soil textures (16 – 66% clay), such biodiversity loss was strongly correlated with the destabilization of soil structure across LUC, whereas soil texture was not so. These observations consistently indicate that the abundance of detritivore soil animals, especially earthworms and termites, may be a significant predictor of soil structure transformations across LUC in tropical environments. Moreover, the sharp reduction in the abundance of earthworms was strongly positively correlated with the decrease in intra macroaggregate-associated C. As a result, after more than 20 years of sugarcane crop there were losses of 40 and 35% of C and N stocks, respectively, resulting in a rate of C emission of $1.3 \text{ Mg ha}^{-1} \text{ yr}^{-1}$. This C loss primarily occurred in the macroaggregate-associated C, as a result of the faster macroaggregate turnover under SC. In summary, the results presented here provide a mechanistic explanation as to why there is soil C depletion when increasing land use intensity in tropical soils: the huge reduction in the abundance of soil engineering invertebrates after LUC impair the ability of soil to physically protect SOM from decomposition inside stable aggregates, and therefore it is a primary mechanism controlling the soil C stocks' depletion across LUC.

Keywords: Aggregation; Carbon storage; Ecosystem functioning; Macrofauna; Soil biodiversity; Soil structure; Sugarcane expansion

1 INTRODUCTION

Globally land use change (LUC) associated with the expansion of biofuel production has impacts on soil organic matter (SOM) (ANDERSON-TEIXEIRA et al., 2009; DON; SCHUMACHER; FREIBAUER, 2011), the largest terrestrial carbon pool (LAL, 2004) and key to the accurate assessment of the CO₂ balance of energy crops (FARGIONE et al., 2008; DJOMO; CEULEMANS, 2012; MELLO et al., 2014). Recent studies have assessed the impacts of converting native ecosystems or cropland into a range of biofuel crop production on soil organic carbon (SOC) dynamics, with attention to its ecosystem services (ANDERSON-TEIXEIRA et al., 2009; DON; SCHUMACHER; FREIBAUER, 2011; FRAZAO et al., 2013; KWON et al., 2013; ZATTA et al., 2014). The recent transformation of pasturelands to sugarcane for ethanol fuel production in Brazil provide a unique opportunity to studies assessing the effects of LUC with increasing land use intensity to SOM storage. Roughly one-third of the global ethanol fuel production has been provided from Brazilian sugarcane, with small contributions from other Latin America countries (GOLDEMBERG et al., 2014). The recent expansion of bioenergy crops in Brazil is driven by an increased demand for ethanol with more than 3 Mha of new sugarcane areas established between 2000 and 2010 in Central-Southern Brazil (ADAMI et al., 2012). It is estimated that this region has concentrated 99% of the recent sugarcane expansion in Brazil in recent years (SPAROVEK et al., 2009; HERNANDES; BUFON; SEABRA, 2014), and approximately 70% of this expansion came from pastures (ADAMI et al., 2012). More than 6.4 Mha of additional sugarcane land will be required to meet the Brazilian demand for ethanol in 2021, with the potential to meet global demand for renewable fuels (GOLDEMBERG et al., 2014).

This intensification of sugarcane expansion can result in initial decreases in SOC stocks when pastures are converted into sugarcane (MELLO et al., 2014) or from native ecosystems to sugarcane (FARGIONE et al., 2008), even though native transitions directly into sugarcane have historically accounted for less than 1% of the sugarcane expansion in Brazil (ADAMI et al., 2012). Although we can accurately assess the effects of LUC on soil carbon stocks with the recent publication of LUC factors for sugarcane production in Brazil (MELLO et al., 2014), the ultimate factors controlling the changes in soil carbon stocks due to sugarcane expansion remain unknown.

The close relationship between soil structural stability and the capacity of soil to stabilize SOM plays a key role in the terrestrial C dynamics (SIX; ELLIOTT; PAUSTIAN, 2000a; SIX et al., 2002). Soil structure is defined as the size and arrangement of particles and

pores in soil (HARTGE; STEWART, 1995), and it regulates a large number of ecological functions, including those that control water infiltration, percolation and retention (CONNOLLY, 1998), gas exchanges (PLAZA-BONILLA; CANTERO-MARTINEZ; ALVARO-FUENTES, 2014), SOM and mineral nutrients dynamics (TISDALL; OADES, 1982; FONTE et al., 2014), soil microbial biomass, diversity and activity (VANGESTEL; MERCKX; VLASSAK, 1996), and the susceptibility of soil to erosion (BARTHES; ROOSE, 2002). Soil structure is the ecosystem property most frequently evaluated when determining soil quality under different land uses (MONCADA et al., 2014) because it is a key factor in the ability of soil to support plant and animal life (BRONICK; LAL, 2005). Both positive and negative effects of LUC and intensification of land use on soil structural quality have been reported (BARTO et al., 2010; SPOHN; GIANI, 2011; CHRENKOVA et al., 2014), and it is still unclear which is the dominating factor of those soil structure responses to LUC in highly weathered tropical soils, which cover 60-70% of tropical areas (SEGALEN, 1994).

Soil structure can be altered directly by management strategies that disturb the soil, or indirectly through impacts on biotic and abiotic factors that affect soil stability (BARTO et al., 2010). However, considerable uncertainty remains with regard to the relative importance of biogenic and abiogenic processes to soil aggregation in tropical soils (PENG et al., 2015). While some studies have emphasized the key role of biogenic processes involving SOM in the structural stabilization of highly weathered soils (VRDOLJAK; SPOSITO, 2002; BARTHES et al., 2008; RABBI et al., 2015), others have pointed abiogenic factors related to the soil texture and clay mineralogy to play a dominant role in aggregation of such soils (SIX; ELLIOTT; PAUSTIAN, 2000b; BARTO et al., 2010).

Although the important role of different groups of soil macrofauna (i.e. soil invertebrates larger than 2 mm) in controlling soil structure dynamic has been demonstrated (CAMMERAAT; RISCH, 2008; JOUQUET et al., 2011; BLOUIN et al., 2013), it remains largely neglect by soil scientists working on soil structure (BOTTINELLI et al., 2015). Soil macroinvertebrates influence soil structure through the incorporation of fresh organic matter in the soil, as this incorporation has large consequences for soil structure because SOM is one of the key factors controlling soil porosity and soil aggregate formation and stability (TISDALL; OADES, 1982). Barros et al. (2001) provided evidences of the role of macrofauna in the transformation and reversibility of soil structure across LUC in tropical soils. It is worth noting that when soil texture is described to be more important than biotic factors for structuring soils, commonly only microbial properties are taken into consideration (e.g. BARTO et al., 2010).

Soil animals are estimated to represent as much as 23% of the total described global diversity (DECAENS et al., 2006), yet remarkably few studies have investigated the effect of large-scale bioenergy crop cultivation on belowground biodiversity and its associated ecosystem functions (ROWE; STREET; TAYLOR, 2009; MCCORMACK et al., 2013). Soil macrofauna provide ecosystem services (WAGG et al., 2014), and are clearly key mediators of soil ecosystem functions for a diversity of ecosystem engineering processes (LAVELLE et al., 2006). The soil engineering concept essentially makes reference to the ability of these organisms to move through the soil and to build biogenic structures with specific physical, chemical and microbiological properties (JOUQUET et al., 2006). Soil macroinvertebrates actively influence pedological processes through structural and mineralogical transformations (DE OLIVEIRA et al., 2014), and improve soil hydraulic properties such as aeration and drainage (LEONARD; RAJOT, 2001). These animals significantly influence nutrient cycling processes (DE VRIES et al., 2013; LUBBERS et al., 2013; WAGG et al., 2014). Moreover, these animals also have an acknowledged role in building and stabilizing soil aggregates with direct implications on flood and erosion control (SIX et al., 2004; BRUSSAARD; DE RUITER; BROWN, 2007), and on soil carbon storage and net soil greenhouse-gas emissions (LUBBERS et al., 2013; MAJEEED et al., 2014). Some species feed on litter and organic residues on the soil surface. They incorporate these organic matters in the soil profile within soil aggregates or in coating their galleries (BOTTINELLI et al., 2015).

LUC associated with bioenergy feedstock production appears a key driver of above and belowground biodiversity changes due to the demand for biofuels, and the negative impacts are primarily reported from tropical regions (MCCORMACK et al., 2013; DESIREE et al., 2014). The existing literature does not show a clear position about the effects of direct LUC for sugarcane expansion on biodiversity (GOLDEMBERG; COELHO; GUARDABASSI, 2008; VERDADE et al., 2012), and particularly the responses of soil biodiversity to these LUC remain unexplored. There is an increase in agriculture intensity with the sugarcane crop establishment over pasturelands. Every five years a cultivation cycle is carried in sugarcane fields with ploughing and fertilization for planting of new stem cuttings, reducing soil carbon stocks over time (MELLO et al., 2014) and potentially altering soil structure and bulk density. In addition, a common management practice used in sugarcane farming in Brazil is the application of significant amounts of organic waste products from ethanol production to the soil under sugarcane cultivation in the form of organic fertilizers (CARVALHO et al., 2013). Sugarcane farming is also characterized by significant inputs of persistent herbicides and controlled release pesticides (BELL; STIRLING; PANKHURST,

2007). Thus, the quality and quantity of inputs available as well as the soil micro-habitats are greatly modified with sugarcane establishment, potentially causing soil biodiversity loss and simplification of the soil community, as it has been observed in other agroecosystems (CALLAHAM et al., 2006; HERNANDEZ-RUIZ; CASTANO-MENESES, 2006; CASTELLANOS-NAVARRETE et al., 2012), reducing the soil food web connections, strongly affecting nutrients cycling (DE VRIES et al., 2013), and impairing ecosystem multifunctionality and sustainability (WAGG et al., 2014).

Land use transitions in sugarcane expansion areas are therefore expected to cause reductions in abundance of individuals and simplification of the macrofaunal community. Such biodiversity loss after LUC would decrease soil engineering processes, resulting in destabilization of soil structure and accelerating the rate of macroaggregates formation and degradation (macroaggregate turnover) irrespectively of the soil texture. We then expected that the ability of soil to physically protect SOM from decomposition inside stable aggregates to be impaired, finally depleting SOC stocks across LUC. In order to test these hypotheses, we conducted a field survey in 3 chronosequences of land use comprising native vegetation (NV), pasture (PA), and sugarcane crop (SC) in Central-Southern Brazil. This land use sequence provides a gradient of land use intensity. The objectives of this study were: (i) to investigate the impact of LUC on soil SOM concentration and C and N storage, (ii) to assess the dynamics of new C inputs from C4 plants entering into pasture and sugarcane soils, (iii) to follow the progress of the soil structural stability and macroaggregate turnover modifications across LUC, (iv) to assess the impacts of LUC on the abundance of individuals, taxa diversity and diversity of functional groups of soil macrofauna, (v) to explore the relative importance of soil texture and biotic factors related to soil macrofaunal community for structural stability of highly weathered tropical soils across LUC, (vi) to evaluate the relationship between soil engineering by macroinvertebrates and the SOM allocation in soil aggregates. To meet the objectives the 3 chronosequences were chosen to exhibit contrasting soil textures, while variations in soil texture among land uses at a single chronosequence were avoided.

2 DEVELOPMENT

2.1 Material and methods

2.1.1 Description of the study sites

The study was carried out in the main sugarcane producing region in the world, Central-Southern Brazil. Three study sites were identified representing the Northern, Center, and Southern parts of the Brazilian sugarcane growing region, including the areas where sugarcane expansion is occurring from pastures: Lat_17S, located in the city of Jataí, Southwestern region of Goiás state ($17^{\circ}56'16"S$, $51^{\circ}38'31"W$) with a mean altitude of 800 m; Lat_21S, located in the city of Valparaíso, West region of São Paulo state ($21^{\circ}14'48"S$, $50^{\circ}47'04"W$) with a mean altitude of 425 m; and Lat_23S, located in the city of Ipaussu, South region of São Paulo state ($23^{\circ}05'08"S$, $49^{\circ}37'52"W$), with a mean altitude of 630 m (Figure 1).

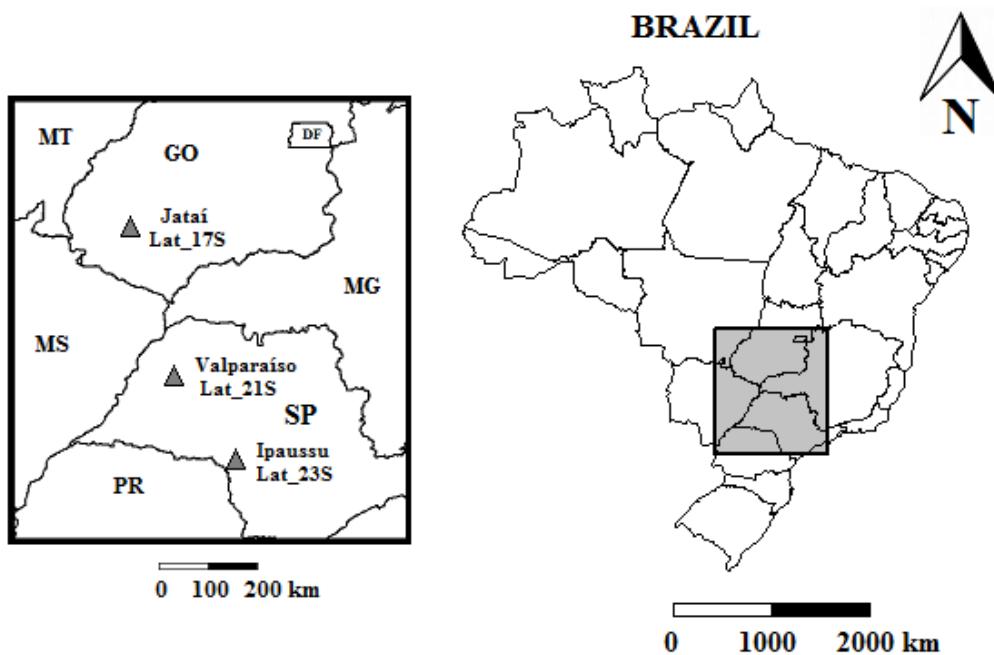


Figure 1 - Geographic location of the study sites across the South-Central sugarcane belt in Brazil

The study sites classified as per Köppen were: Aw (mesothermal tropical) at Lat_17S, where the mean annual temperature (MAT) is 24.0°C and the mean annual precipitation (MAP) is 1,600 mm; Aw (humid tropical) at Lat_21S, where MAT is 23.4°C

and MAP is 1,240 mm; Cwa (tropical) at Lat_23S, where MAT is 21.7 °C and MAP is 1,470 mm. Both three sites present the rainfall season concentrated in the Spring-Summer (October to April) and the dry season in the Autumn-Winter (May to September).

The soils of the study sites were classified according to Soil Survey Staff (2014) (Table 1). The three sites were primarily well-drained and highly weathered surfaces, typical of tropical wet conditions. In each study site we identified a chronosequence of land use for: native vegetation (NV), pasture (PA), and sugarcane crop (SC). In order to minimize the effects of climatic, topographic and edaphic variations, the three land uses were always located in adjacent areas. Table 1 shows the soil bulk density and clay contents in the 0-10 cm, 10-20 cm, and 20-30 cm soil layers, besides the information on land use and management for each field site which includes the type and duration of each land use, as well as nutrient inputs. Briefly, the NV at Lat_17S comprises the Cerradao forest formation, while at Lat_21S and Lat_23S NV comprises a transition between the Atlantic forest and Cerrado vegetation. LUC from NV to PA happened in 1980 at Lat_17S and Lat_21S, and in 1979 at Lat_23S. PA areas differed from each other in the stocking rate: PA at Lat_17S supports 1.5 animal unit (AU) ha⁻¹; PA at Lat_21S supports around 2 AU ha⁻¹; and PA at Lat_23S supports around 1 AU ha⁻¹ along the year. The SC was established over part of PA in 2009 at Lat_17S, in 2010 at Lat_21S, and in 1990 at Lat_23S. The nutrient inputs in SC differed among the sites, with annual inputs of mineral P at Lat_21S and high amounts of organic fertilizers at Lat_23S (Table 1).

Table 1 - Soil classification according to Soil Survey Staff (2014), bulk density (BD), clay content, and the information on land use and management for native vegetation (NV), pasture (PA), and sugarcane crop (SC) in the study sites

Site ^a	Land Use	Soil classification	Soil layer cm	BD g cm ⁻³	Clay g kg ⁻¹	Land use change and management
Lat_17S	NV	Anionic Acrudox	0-10	0.97	311.4	Cerradão forest formation, Cerrado biome, with dense vegetation compared to the Cerrado <i>stricto sensu</i> (savanna).
			10-20	1.01	324.9	
			20-30	0.97	347.1	
	PA	Typic Hapludox	0-10	1.18	147.8	Conversion from NV to PA with tropical grasses of the genus <i>Brachiaria</i> happened in 1980. PA supports 1.5 animal unit (AU) ha ⁻¹ along the year.
			10-20	1.26	150.4	
			20-30	1.29	158.8	
	SC	Anionic Acrudox	0-10	1.26	353.0	Established over part of PA in 2009 by plowing, listing and disking the soil, with limestone application (1.6 ton ha ⁻¹). Fertilized with 450 kg ha ⁻¹ yr ⁻¹ of the formula 22-00-17 (N-P ₂ O ₅ -K ₂ O), and 50 kg ha ⁻¹ of a liquid fertilizer (20% N). Harvested without cane-burning.
			10-20	1.19	347.8	
			20-30	1.22	357.0	
Lat_21S	NV	Typic Rhodudalf	0-10	0.99	186.1	Seasonal semideciduous forest, comprising a transition between the Atlantic forest and Cerrado vegetation.
			10-20	1.08	173.8	
			20-30	1.21	173.6	
	PA	Typic Kandiudult	0-10	1.22	173.1	Conversion from NV to PA occurred in 1980, and PA supports around 2 AU ha ⁻¹ along the year. PA is comprised by grasses of the genus <i>Brachiaria</i> , and was fertilized with 120 kg ha ⁻¹ yr ⁻¹ of the formula 20-05-19.
			10-20	1.34	176.3	
			20-30	1.41	179.0	
	SC	Typic Hapludalf	0-10	1.21	151.2	Established over part of the pasture in 2010, by plowing, listing and disking the soil. SC fertilization is 540 kg ha ⁻¹ yr ⁻¹ of 4-20-20, with a single application of 150 m ³ ha ⁻¹ of vinasse in 2012. Mechanically harvested without cane-burning since planting.
			10-20	1.29	162.6	
			20-30	1.38	162.3	
Lat_23S	NV	Rhodic Hapludox	0-10	0.71	647.4	NV is similar to Lat_21S site, described before.
			10-20	0.83	662.8	
			20-30	0.83	670.0	
	PA	Rhodic Kandiudox	0-10	1.05	572.7	Land use conversion from NV to PA occurred in 1979. PA supports around 1 AU ha ⁻¹ and is composed of tropical grasses of the genus <i>Cynodon</i> spp..
			10-20	1.03	583.3	
			20-30	0.92	615.0	
	SC	Rhodic Hapludox	0-10	1.07	662.3	Established over part of PA in 1990, by the same soil management described for the others sites. Annually, SC fertilization was 200 m ³ ha ⁻¹ of vinasse, 25 ton ha ⁻¹ of filter cake and boiler ash, and 100 kg ha ⁻¹ of ureia. Mechanically harvested without cane-burning since 2003.
			10-20	1.06	641.9	
			20-30	1.06	649.4	

^a Lat_17S, Southwestern region of Goiás state (17°56'16"S, 51°38'31"W); Lat_21S, West region of São Paulo state (21°14'48"S, 50°47'04"W); Lat_23S, South region of São Paulo state (23°05'08"S, 49°37'52"W).

2.1.2 Soil Sampling

The soil sampling was carried out in the rainy season, in January 2013. During the rainy season it is expected to be triggered a hot moment of high soil fauna richness and reduced variation of abundance, which is therefore more indicated for the assessment of soil biodiversity (DA CUNHA NETO et al., 2012). All samples from a single site were taken on the same day. Sampling for each land use site consisted of a 2.25 ha grid with 9 sampling points spaced 50 m apart, composing 27 sampling points for each study site, or 81 sampling points in total (Figure 2). Samples for C and N contents and isotope analyses were taken within a radius of 6 meters around each sampling point. 12 subsamples were collected from 0-10 cm, 10-20 cm, and 20-30 cm soil layers using a soil Dutch auger, and combined (resulting in one sample for each soil depth in each sampling point). Samples were then ground and sieved to 0.150 mm.

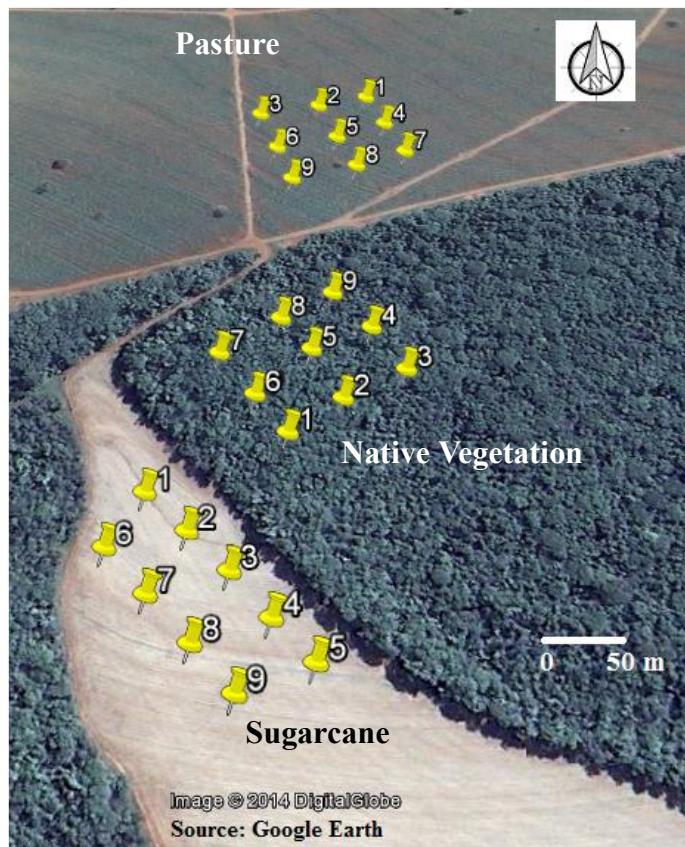


Figure 2 - The chronosequence and sampling points allocation at Lat_23S

Soil blocks of 5 x 5 cm to 10 cm depth were collected from 0-10 cm, 10-20 cm, and 20-30 cm soil layers at each sampling point for soil aggregates analyses. Field moist soil was

gently passed through an 8-mm sieve by breaking up the soil along natural planes of weakness, and air dried.

At each site macrofauna samples were extracted from the 2.25 ha grid with 9 sampling points spaced 50 m apart (Figure 2). Soil blocks of 25 x 25 cm to 10 cm depth were collected from 0-10 cm, 10-20 cm, and 20-30 cm soil layers at each sampling point, and sorted according to the standard Tropical Soil Biology and Fertility Institute (TSBF) soil monolith method (ANDERSON; INGRAM, 1993; MOREIRA; HUISING; BIGNELL, 2008). In total 81 monoliths were excavated. The animals were carefully hand-sorted from the soil blocks in a large tray, immediately after the sampling procedure. Organisms from the litter were added with the 0-10 cm soil macrofauna. The earthworms were preserved in 92.8% ethanol and all the others individuals in 70% ethanol for subsequent laboratory identification and counting. The invertebrates were sorted into the taxonomic groups: Aranae, Blattodea, Chilopoda, Coleoptera, Dermaptera, Diplopoda, Diptera, Formicidae, others Hymenoptera, Gastropoda, Hemiptera, Isopoda, Isoptera, Oligochaeta, and Scorpiones.

2.1.3 Analyses

Organic carbon and total nitrogen were determined by dry combustion on elemental analyzer – LECO® CN-2000 (furnace at 1350 °C in pure oxygen). Isotope composition of C and N was determined by using a Thermo Quset-Finnigan Delta Plus isotope ratio mass spectrometer (Finnigan-MAT) interfaced to an Elemental Analyzer (Carlo Erba). Isotope ratios are expressed in the classical δ -notation with respect to the Vienna Pee Dee Belemnite (V-PDB) standard. Reproducibility of the determinations is better than 0.2‰ for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$.

For the soil structure study, two pretreatments were applied before wet sieving, according to the method from Six et al. (2000b): air-dried soil was rapidly immersed in water (slaked), and air-dried soil was capillary rewetted to field capacity and equilibrated overnight before immersion in water (rewetted treatment). In use both pretreatments, we avoid physical induced variability due to the seasonal differences in water content, which is not related to soil quality (SIX; ELLIOTT; PAUSTIAN, 2000b). The soils were then separated into four fractions using a method adapted from Elliott (1986) in order to determine aggregate stability and distribution among aggregate size fractions: (i) large macroaggregates (LM, $\geq 2000 \mu\text{m}$), (ii) small macroaggregates (SM, 250-2000 μm), (iii) microaggregates (Mi, 53-250 μm), and (iv) silt + clay sized particles (S+C, $\leq 53 \mu\text{m}$). Briefly, 50g of soil (slaked or rewetted) were submerged in deionized water on the top of a set of three sieves (2000, 250, and 53 μm) and

oscillated with a displacement of 4 cm at 30 cycles per minute for 10 min. Aggregates remaining on the sieves were then rinsed into a pre-weighted plastic pan for oven dry at 50 °C, and weighted. The sum of the weights of LM, SM, and Mi was subtracted from the initial weight of the sample (50g) to obtain the total amount of the S+C fraction ($\leq 53 \mu\text{m}$). To avoid overestimating the aggregate fractions masses, the sand size distribution for LM, SM and Mi of rewetted and slaked aggregates was determined by sieving after dispersing the aggregates with sodium hexametaphosphate (5 g L⁻¹), and the aggregate-sized sand fraction was subtracted from the whole fractions masses.

The large and small macroaggregate fractions were then combined and used for the isolation of: inter-microaggregate particulate organic matter (POM; $\geq 250 \mu\text{m}$), microaggregates within macroaggregates (53-250 μm), and silt and clay ($\leq 53 \mu\text{m}$). The macroaggregate fractionation was carried out with a device described by Six et al. (2000a) which completely breaks up macroaggregates with minimal disruption of microaggregates. About 10g of the oven-dried macroaggregates was pre-slaked overnight in deionized water in order to slake the rather stable macroaggregates of the tropical weathered soils. The macroaggregates were then transferred to the device holding a 250 μm mesh screen and shaken with 50 glass beads (diameter 4 mm) until all macroaggregates were broken up. The microaggregates released were immediately flushed through the 250 μm sieve and deposited onto a 53- μm sieve by a continuous flow of deionized water through the device. The material on the 53 μm sieve was then wet-sieved to separate the stable microaggregates from the silt and clay. Once all the macroaggregates were broken up, all fractions retained on the 250 μm mesh screen, and silt and clay were backwashed into a pre-weighed container. All fractions were oven-dried at 50 °C for 48 h, weighed and stored. The C analyses of the macroaggregate fractions were carried out as described above for the whole soil samples.

The microaggregates within macroaggregates fraction (53-250 μm) was used for the assessment of the SOM humification degree through Laser-Induced Fluorescence Spectroscopy (LIF) using the methods described by Milori et al. (2006). Briefly, approximately 0.5g of microaggregates within macroaggregates, after being grinded to pass a 250- μm mesh, were pressed into pellets of 1-cm diameter and 2-mm thickness, which were then inserted into a bench custom-made apparatus in order to run LIF measurements. Samples were excited with 458 nm blue radiation, emitted by argon laser equipment with power of around 300 mW. The ratio of the area of the LIF spectrum of each sample over the corresponding C concentration was defined as the SOM humification index (HLIF) and is expressed as arbitrary units (a.u.).

2.1.4 Calculations and data analyses

The C and N stocks were calculated for each soil layer by multiplying the content of each one by the soil bulk density and the layer thickness (10 cm). Afterwards, the stocks were calculated for each sampling point, and finally for the site. To account for the effect of differing soil bulk densities (due to land use change) on stocks comparisons, the stocks within the pasture and sugarcane soils were adjusted to an equivalent soil mass basis from the soil mass under the corresponding NV (LEE et al., 2009).

The proportion of new carbon added from C4 plants (pasture and sugarcane) compared to the C3 plants (NV) was estimated using the results of the natural abundance of $\delta^{13}\text{C}$ (VITORELLO et al., 1989). The amount of C derived from C3 plants ($C_{\text{C}3}$) and from C4 plants ($C_{\text{C}4}$) for pasture and sugarcane soils were calculated according to equations (CERRI et al., 2004):

$$C_{\text{C}4} = C_t \cdot (\delta^{13}\text{C}_{\text{C}4} - \delta^{13}\text{C}_{\text{C}3}) / (\delta^{13}\text{C}_{\text{plant}} - \delta^{13}\text{C}_{\text{C}3}), \quad C_{\text{C}3} = C_t - C_{\text{C}4}$$

$$\text{PC}_{\text{C}4} = 100 \cdot (\delta^{13}\text{C}_{\text{C}4} - \delta^{13}\text{C}_{\text{C}3}) / (\delta^{13}\text{C}_{\text{plant}} - \delta^{13}\text{C}_{\text{C}3}), \quad \text{PC}_{\text{C}3} = 100 - \text{PC}_{\text{C}4}$$

where C_t is the total C content of the pasture or sugarcane soil layer, $\delta^{13}\text{C}_{\text{C}4}$ is the $\delta^{13}\text{C}$ value of the respective pasture or sugarcane soil layer, $\delta^{13}\text{C}_{\text{C}3}$ is the $\delta^{13}\text{C}$ value of the corresponding NV soil layer and $\delta^{13}\text{C}_{\text{plant}}$ is the $\delta^{13}\text{C}$ average value for pasture (-13‰) or sugarcane (-15‰). $\text{PC}_{\text{C}4}$ and $\text{PC}_{\text{C}3}$ are the percent of total soil C from C4 and C3 vegetation, respectively.

The rates of C sequestration or emission for the land use changes from NV to pasture and from pasture to sugarcane were estimated for each study site using the equations:

$$\text{Rate}_{(\text{NV/P})} = (C_p - C_{\text{NV}}) / T$$

$$\text{Rate}_{(\text{P/SC})} = (C_{\text{SC}} - C_p) / T$$

where $\text{Rate}_{(\text{NV/P})}$ is the rate for the land use change from NV to pasture, $\text{Rate}_{(\text{P/SC})}$ is the rate for the land use change from pasture to sugarcane, C_p is the C stock under pasture, C_{NV} is the C stock under NV, C_{SC} is the C stock in sugarcane soil, and T is time period since land use was changed: 33, 33, and 34 years since conversion of NV to pasture, and 4, 3, and 23 years since conversion of pasture to sugarcane at Lat_17S, Lat_21S and Lat_23S, respectively.

The faunal density was calculated as the number of individuals per surface unit (m^2). Faunal diversity was assessed by richness (number of taxa), and the Shannon's diversity index (H'), and Pielou's evenness index (E) (ODUM, 1983).

The taxa were classified according to their ecological functions as herbivorous (Dermoptera and Hemiptera), detritivorous (Blattodea, Diplopoda, Diptera, Gastropoda, Isopoda, Isoptera and Oligochaeta) or predators (Aranae, Chilopoda, Coleoptera, Formicidae, Hymenoptera and Scorpiones) (BROWN et al., 2001; JONES et al., 2010), although we recognize that categorize the animals according to their feeding habits in all faunal groups is imprecise due the presence of more than one ecological function in the same taxa (ROBERTSON; KETTLE; SIMPSON, 1994).

We calculated the man weight diameter (MWD) for the slaked and rewetted soils as a preliminary index of aggregate stability. The normalized stability index (NSI) was then used as the real index of soil structural stability because it eliminates the confounding effects of soil pretreatment, corrects for differences in sand size distribution among soils, aggregate size classes and pretreatments, and normalizes the level of disruption imposed by slaking by using a maximum level of disruption (SIX; ELLIOTT; PAUSTIAN, 2000b). NSI was measured according the calculation procedures described by Six et al. (2000b). However, we used no weighting factors in the calculation of both disruption level and maximum disruption level. We argue that the arbitrary weighting factors for the disruption in different aggregate size classes make the index insensible to the preferential disaggregation of the larger size classes after soil disturbing as observed in highly weathered tropical soils.

Comparisons between land use types (NV, PA and SC) were carried out for SOM and soil aggregation variables using one-way ANOVA with land use type as the main factor and sites considered as blocks and treated as a random variable. Data transformations were not necessary to meet the assumptions of ANOVA. A Scott-Knott test was used to prove significant differences among land uses. All analyses were conducted using the software R, version 3.1.0 (TEAM, 2014) and significance level was set at $p < 0.05$.

The biological data (total soil macrofauna density, group richness, Shannon index, evenness and the functional groups – predators, herbivorous and detritivorous) were submitted to the Shapiro-Wilk normality test. To assess land use effects, the variables that attended to the normality distribution were submitted to an ANOVA, followed by Fisher's Protected LSD test, and the variables that did not attend to the normality, after trying data transformation, were submitted to the Kruskal-Wallis test. All analyses were conducted using the software Statistica 7.0 (STATSOFT, 2004).

The functional groups' data were used to multivariate analysis, being used to obtain the gradient length (DCA). Because this length was smaller than three (linear response), a Principal Component Analysis (PCA) was performed using CANOCO version 4.5 (TER

BRAAK; ŠMILAUER, 2002) to visualize the distribution of the land uses in relation to the ecological functions. This same analysis was carried out to visualize the relationships between macrofauna biodiversity and soil structural stability.

2.2 Results

2.2.1 LUC effects on soil organic matter levels

There were significant losses of SOM due to LUC (Table 2). In a regional scale, soil C and N contents significantly decreased from NV to PA at all depths. Both elements also decreased from PA to SC, with significant effects in the upper 10 cm of soil. Overall, there were 37% lower soil C and 43% lower N contents in SC soils compared to NV soils (Table 2).

Statistical differences between land uses at an individual site cannot be described because there is no replication of land uses at each site. However, consistent responses were identified when each site was addressed individually. The largest SOM losses occurred at Lat_21S and Lat_23S, with an overall reduction of 54% and 52% of the C and N content, respectively (Table 2).

2.2.2 Isotopic abundance of $\delta^{15}\text{N}$ and SOM humification degree

Significant differences were not revealed in $\delta^{15}\text{N}$ signatures (Table 3). The mean values for $\delta^{15}\text{N}$ ranged from 7.6‰ for NV soils to 7.2‰ for PA and 8.0‰ for SC soils. The lowest value for $\delta^{15}\text{N}$ (4.7‰) was found in the upper 10 cm of NV at Lat_17S, and the highest value (10.2‰) occurred in the deepest layer of SC soil at Lat_23S. The $\delta^{15}\text{N}$ signatures showed a pronounced overall increase in ^{15}N enrichment with increasing soil depth in all land uses and field sites investigated (Table 3).

Table 2 - Contents of soil organic carbon and nitrogen, in 0-10, 10-20, and 20-30 cm soil layers under native vegetation (NV), pasture (PA), and sugarcane crop (SC). Standard error of the mean is presented in parenthesis. Letters represent statistically significant differences between land uses according Scott-Knott test

Depth cm	Organic C			Total N		
	NV	PA ----- g kg ⁻¹ -----	SC	NV	PA ----- g kg ⁻¹ -----	SC
<i>Lat_17S^a</i>						
0-10	15.6 (0.5)	9.5 (0.3)	10.8 (0.4)	1.2 (0.1)	0.7 (<0.1)	0.8 (0.1)
10-20	12.9 (0.3)	8.4 (0.4)	10.4 (0.4)	1.0 (<0.1)	0.5 (<0.1)	0.7 (0.1)
20-30	10.7 (0.5)	6.4 (0.2)	9.7 (0.3)	0.9 (<0.1)	0.5 (<0.1)	0.6 (0.1)
<i>Lat_21S</i>						
0-10	21.8 (0.9)	13.3 (0.5)	11.1 (0.4)	2.2 (0.1)	1.1 (0.1)	1.1 (<0.1)
10-20	16.0 (0.8)	9.5 (0.2)	9.9 (0.2)	1.7 (0.1)	0.8 (<0.1)	1.0 (<0.1)
20-30	14.9 (1.9)	7.5 (0.2)	8.0 (0.2)	1.6 (0.2)	0.6 (0.1)	0.8 (<0.1)
<i>Lat_23S</i>						
0-10	36.7 (1.6)	36.4 (1.4)	18.9 (1.0)	3.1 (0.2)	2.6 (0.2)	1.5 (0.1)
10-20	33.7 (1.5)	27.6 (0.8)	18.4 (0.9)	3.0 (0.2)	2.1 (0.1)	1.4 (0.1)
20-30	30.3 (1.3)	20.6 (1.0)	17.1 (0.8)	2.6 (0.1)	1.6 (0.1)	1.5 (<0.1)
<i>Average</i>						
0-10	24.7 (1.0) a	19.8 (0.7) b	13.6 (0.6) c	2.2 (0.1) a	1.5 (0.1) b	1.1 (0.1) c
10-20	20.1 (0.9) a	15.2 (0.5) b	12.9 (0.5) b	1.9 (0.1) a	1.1 (0.1) b	1.0 (<0.1) b
20-30	18.6 (1.3) a	11.6 (0.4) b	11.5 (0.4) b	1.7 (0.1) a	1.0 (0.1) b	0.9 (0.1) b

^a Lat_17S, Southwestern region of Goiás state (17°56'16"S, 51°38'31"W); Lat_21S, West region of São Paulo state (21°14'48"S, 50°47'04"W); Lat_23S, South region of São Paulo state (23°05'08"S, 49°37'52"W)

Table 3 - Isotopic signatures ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) in 0-10, 10-20, and 20-30 cm soil layers under native vegetation (NV), pasture (PA), and sugarcane crop (SC). Standard error of the mean is presented in parenthesis. Letters represent statistically significant differences between land uses according Scott-Knott test

Depth cm	$\delta^{13}\text{C}$			$\delta^{15}\text{N}$		
	NV	PA	SC	NV	PA	SC
<i>Lat_17S^a</i>						
0-10	-25.2 (0.3)	-20.4 (0.2)	-18.0 (0.3)	4.7 (0.2)	5.7 (0.2)	6.9 (0.1)
10-20	-24.2 (0.3)	-20.8 (0.2)	-18.2 (0.3)	5.8 (0.1)	6.3 (0.1)	7.2 (0.2)
20-30	-23.5 (0.4)	-21.1 (0.1)	-18.1 (0.4)	6.9 (0.2)	6.7 (0.1)	7.7 (0.2)
<i>Lat_21S</i>						
0-10	-26.1 (0.2)	-14.4 (0.1)	-16.3 (0.1)	7.6 (0.2)	6.0 (0.1)	6.2 (0.2)
10-20	-26.0 (0.4)	-15.2 (0.1)	-16.7 (0.1)	7.9 (0.1)	7.0 (0.1)	6.9 (0.2)
20-30	-25.9 (0.1)	-16.1 (0.1)	-17.7 (0.2)	8.4 (0.1)	7.7 (0.1)	7.7 (0.2)
<i>Lat_23S</i>						
0-10	-25.2 (0.1)	-14.8 (0.1)	-18.7 (0.5)	9.0 (0.3)	7.4 (0.2)	9.2 (0.3)
10-20	-25.3 (0.1)	-15.8 (0.2)	-19.0 (0.4)	9.2 (0.2)	8.6 (0.2)	9.9 (0.2)
20-30	-25.2 (0.1)	-17.3 (0.2)	-19.5 (0.4)	9.3 (0.3)	9.8 (0.3)	10.2 (0.2)
<i>Average</i>						
0-10	-25.5 (0.2) b	-16.5 (0.1) a	-17.7 (0.3) a	7.1 (0.2) a	6.4 (0.2) a	7.4 (0.2) a
10-20	-25.2 (0.2) b	-17.3 (0.1) a	-18.0 (0.3) a	7.6 (0.2) a	7.3 (0.1) a	8.0 (0.2) a
20-30	-24.9 (0.2) b	-18.1 (0.1) a	-18.4 (0.3) a	8.2 (0.2) a	8.1 (0.2) a	8.5 (0.2) a

^a Lat_17S, Southwestern region of Goiás state (17°56'16"S, 51°38'31"W); Lat_21S, West region of São Paulo state (21°14'48"S, 50°47'04"W); Lat_23S, South region of São Paulo state (23°05'08"S, 49°37'52"W)

2.2.3 Sugarcane expansion and implications for C and N storage

The SOC stocks tended to be higher under NV at all field sites (Figure 3). The conversion of NV to PA decreased SOC stocks from 38.7 to 25.8 Mg ha⁻¹ at Lat_17S (Figure 3a), from 56.8 to 35.2 Mg ha⁻¹ at Lat_21S (Figure 2b), and from 78.6 to 72.2 Mg ha⁻¹ at Lat_23S (Figure 3c). Overall, SOC stocks decreased at an average rate of 0.4 Mg ha⁻¹ yr⁻¹ after the conversion of NV to PA (Figure 4a).

Isotopic analyses showed significant enrichment in ¹³C from NV to PA at all soil depths (Table 3). When quantified the replacement of the original SOM (C_{C3}) with new organic matter added by C4 plants (C_{C4}), at all three sites was evidenced a rapid loss of C_{C3}, which was only partially offset by the introduction of new C_{C4} (Figure 4a). There was 31% C_{C4} under PA at Lat_17S, 82% C_{C4} at Lat_21S, and 76% C_{C4} at Lat_23S (Figure 3).

SOC stock depletion was also observed in conversion of PA to SC, with an average loss rate of 0.25 Mg ha⁻¹ yr⁻¹ (Figure 4b). Sugarcane soils had similar or larger SOC stocks than pastures where this land use transition had less than 5 years (Figure 3a,b), while SOC stock losses of 29.1 Mg ha⁻¹ were observed at Lat_23S with more than 20 years of sugarcane crop (Figure 3c).

There was an increase at a rate of 1.3 Mg ha⁻¹ yr⁻¹ at Lat_17S (Figure 4b). In this specific site the replacement of the original C_{C3} doubled from 31% C_{C4} under pasture to 66% C_{C4} in sugarcane soil (Figure 3a), and the annual introduction of new C_{C4} overcomes the losses of original C_{C3}. At Lat_21S and Lat_23S, on the other hand, there were SOC stock losses and C_{C4} depletion as well, with the largest C loss rate of 1.3 Mg ha⁻¹ yr⁻¹ at Lat_23S (Figure 4b). This C loss at Lat_23S was promoted by the respiration of C_{C4}.

Total mean N storage in soil significantly decreased from 5.3 Mg ha⁻¹ in NV to 3.3 Mg ha⁻¹ in PA (Figure 5b). The conversion of NV to PA decreased N stocks consistently at all sites (Figure 5a), even at Lat_21S where there were annual inputs of N fertilizers in PA (Table 1). N stocks reduction from NV to PA ranged from 3.0 Mg ha⁻¹ to 1.7 at Lat_17S, from 6.0 Mg ha⁻¹ to 3.0 at Lat_21S, and from 6.8 Mg ha⁻¹ to 5.3 Mg ha⁻¹ at Lat_23S.

There were no significant differences in mean N stocks between PA and SC soils (Figure 5b), despite a tendency of higher values under SC at Lat_17S and Lat_21S (Figure 5a), with less than 5 years of land use transition. At Lat_23S, however, more than 20 years of sugarcane crop caused a reduction of 1.9 Mg ha⁻¹ in N stock compared to the pasture soil.

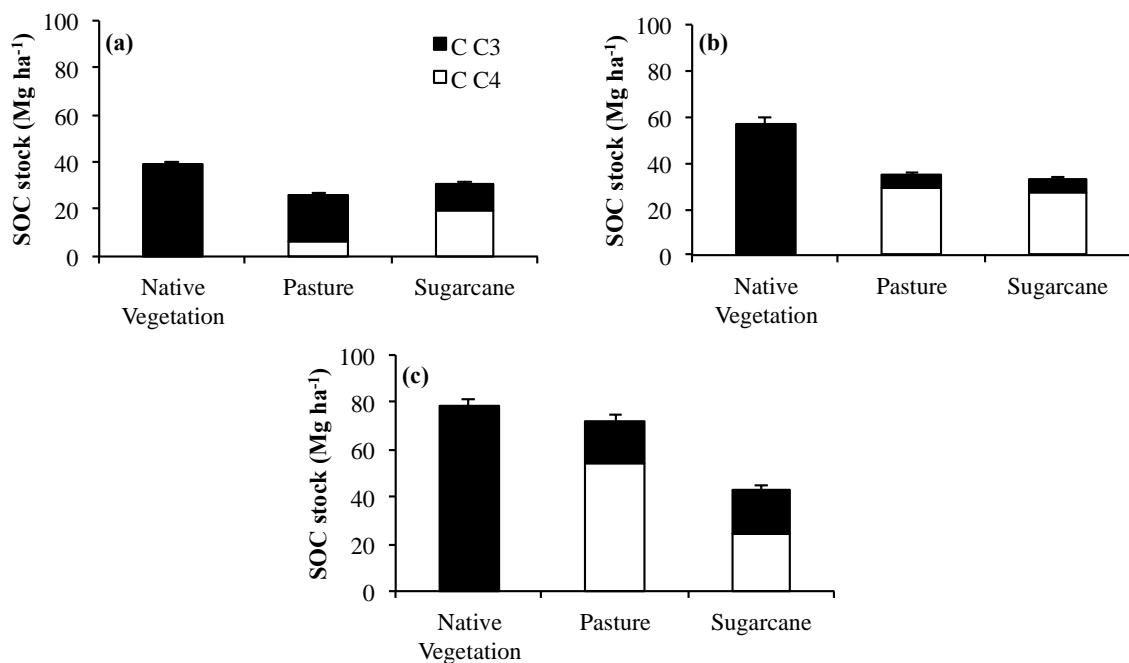


Figure 3 - Soil carbon stocks and its origin in 0-30 cm soil layer as a function of the land use change at Lat_17S (a), Lat_21S (b), and Lat_23S (c). Error bars denote standard error of the mean

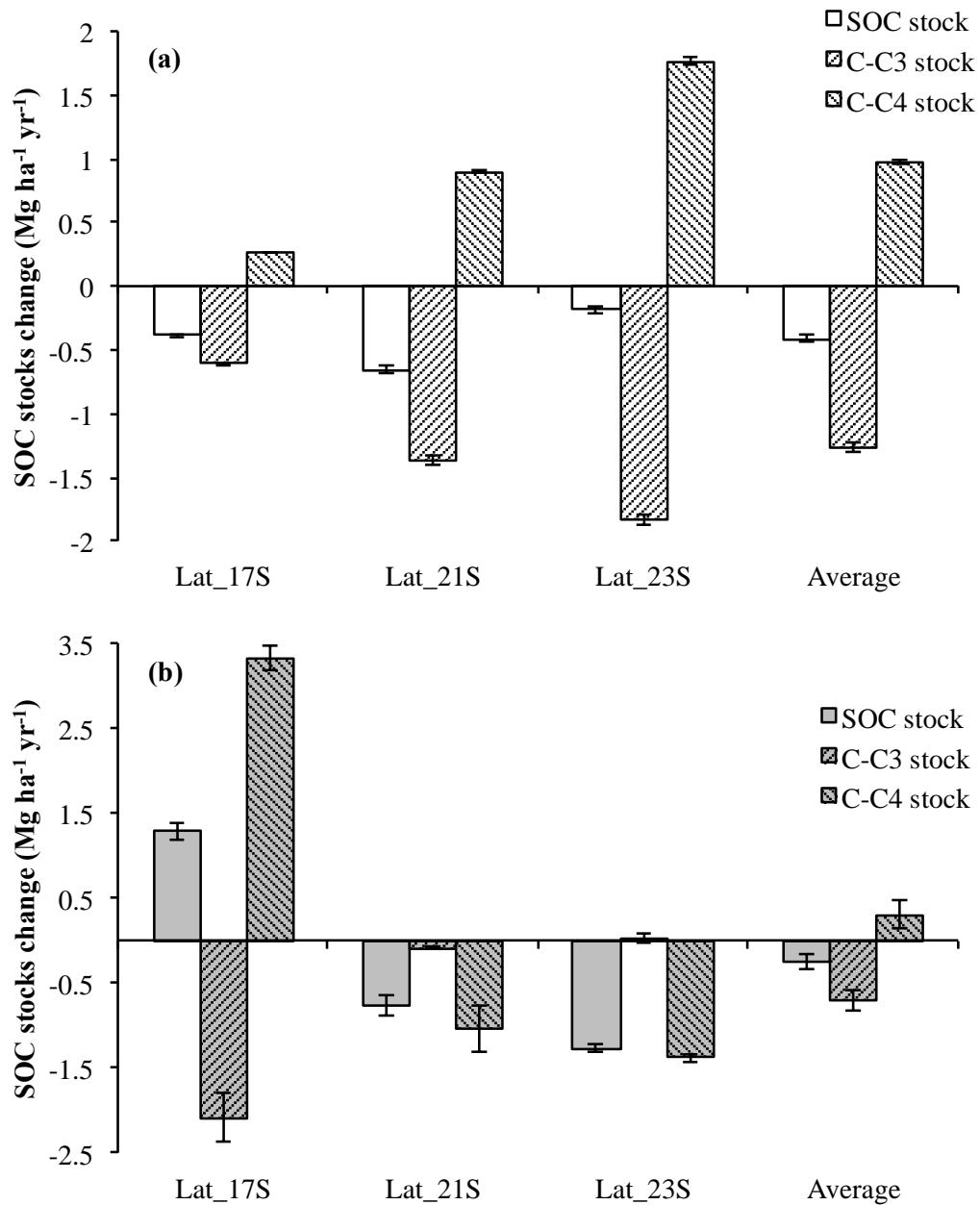


Figure 4 - Average annual changes in the soil organic carbon (SOC) stocks in 0-30 cm soil layer as a function of the land use change from native vegetation to pasture (a) and from pasture to sugarcane (b) in three study sites at the South-Central Brazil. Error bars denote standard error of the mean

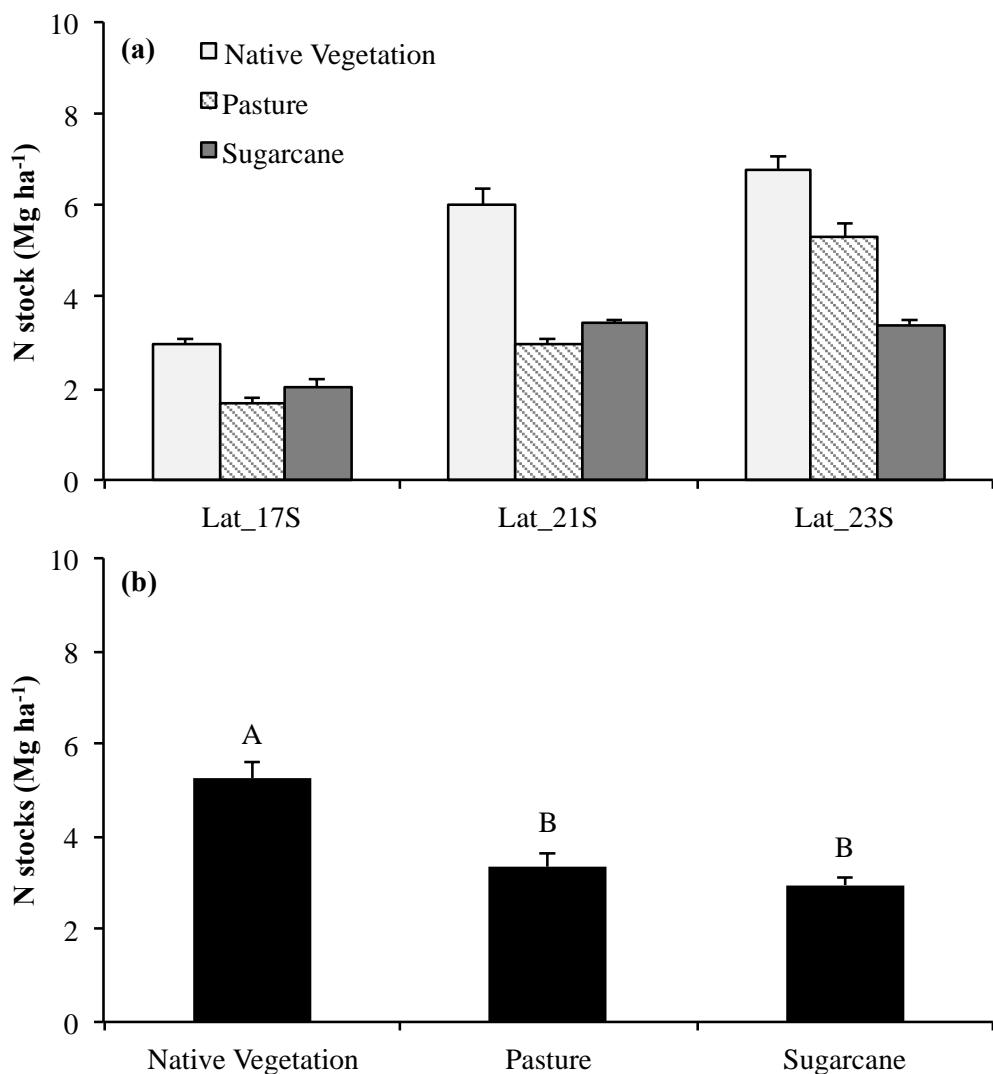


Figure 5 - Soil total nitrogen stocks in 0-30 cm soil layer as a function of the land use change at Lat_17S, Lat_21S, and Lat_23S (a), and the average of the three sites (b). Error bars denote standard error of the mean. Letters represent statistically significant differences between land uses according Scott-Knott test

2.2.4 Size distribution of soil aggregates

The aggregate-size distribution for slaked soil is present because the capillary-wetted aggregate distribution is not suitable to show effects of different land uses (SIX; ELLIOTT; PAUSTIAN, 2000b). The distributions of slaked soil among the different aggregate-size classes were modified by LUC (Table 4). Water-stable macroaggregates (250-200 μm plus >2000 μm) dominated the aggregate-size distribution at all field sites and land uses. This fraction on average accounted for 87, 91 and 77% of the dry soil aggregates weight in NV, PA and SC soils, respectively, with greatest proportions at Lat_23S and lowest at Lat_21S (Figure 6). The macroaggregates were major consisted by large macroaggregates (>2000 μm) under NV and pasture, and by small macroaggregates (250-2000 μm) in SC soils. The conversion from NV to pasture increased large macroaggregates and decreased small macroaggregates proportions at Lat_17S and Lat_23S sites, and this land use transition mostly decreased microaggregates (53-250 μm) (Table 4).

The conversion of pasture to SC decreased large macroaggregates and increased small macroaggregates and microaggregates at all field sites, resulting in higher proportions of small macroaggregates and microaggregates in SC soils compared to NV and PA (Table 4 and Figure 6). The proportion of silt plus clay fraction (<53 μm) was lower in pasture soils at Lat_17S and Lat_23S. A clear trend of aggregate-size distribution with soil depth was verified only in NV soils, with reductions in large macroaggregates, and increases in small macroaggregates and microaggregates with increasing depth (Table 4).

2.2.5 Mean weight diameter

Land use conversion from NV to pasture increased MWD for 0-30 cm layer upon slaked at Lat_17S (from 3.32 to 4.08 mm) and Lat_23S (from 3.84 to 4.72 mm) (Figure 7a). At Lat_21S, similar results for this land use transition were observed in 10-20 and 20-30 cm soil depth, with increases from 2.71 to 3.84 mm and from 2.51 to 3.19 mm, respectively (Table 5). From pasture to sugarcane, MWD upon slaked decreased at all field sites (Figure 7a). These results were consistently observed in all soil depths (Table 5). Largest values for each land use were found at Lat_23S. On the whole, capillary-wetted soil MWD showed similar trends across land uses compared to slaked soil MWD (Figure 7b).

Table 4 - Aggregates size distribution at 0-10, 10-20, and 20-30 cm depths for slaked soils of native vegetation (NV), pasture (P), and sugarcane (SC) areas

cm	< 53 µm			CV	53-250 µm			CV	250-2000 µm			CV	> 2000 µm			CV
	NV	P	SC		NV	P	SC		NV	P	SC		NV	P	SC	
<i>Lat_17S^a</i>																
0-10	0.04	0.01	0.04	38.11	0.05	0.06	0.23	44.35	0.22	0.16	0.60	21.67	0.69	0.77	0.13	12.82
10-20	0.04	0.02	0.03	35.78	0.07	0.05	0.19	37.30	0.31	0.16	0.69	19.79	0.57	0.78	0.10	17.84
20-30	0.04	0.01	0.03	46.09	0.08	0.05	0.13	41.87	0.37	0.14	0.72	19.38	0.52	0.80	0.13	15.30
<i>Lat_21S</i>																
0-10	0.02	0.02	0.03	34.18	0.06	0.12	0.37	31.93	0.07	0.13	0.25	41.27	0.85	0.73	0.35	12.29
10-20	0.02	0.02	0.03	75.52	0.14	0.12	0.22	41.37	0.25	0.13	0.28	61.12	0.48	0.74	0.42	29.85
20-30	0.01	0.01	0.02	26.75	0.27	0.17	0.25	55.81	0.29	0.24	0.41	59.66	0.43	0.58	0.32	47.17
<i>Lat_23S</i>																
0-10	0.04	0.04	0.04	43.82	0.02	<0.01	0.08	62.22	0.09	0.01	0.33	52.18	0.85	0.94	0.51	15.00
10-20	0.03	0.02	0.04	52.65	0.03	0.01	0.13	55.30	0.17	0.02	0.48	24.18	0.76	0.95	0.35	11.96
20-30	0.04	0.02	0.04	50.98	0.05	0.01	0.08	58.61	0.30	0.05	0.49	41.58	0.62	0.92	0.39	23.79

^a Lat_17S, Southwestern region of Goiás state (17°56'16"S, 51°38'31"W); Lat_21S, West region of São Paulo state (21°14'48"S, 50°47'04"W);

Lat_23S, South region of São Paulo state (23°05'08"S, 49°37'52"W).

CV, coefficient of variation.

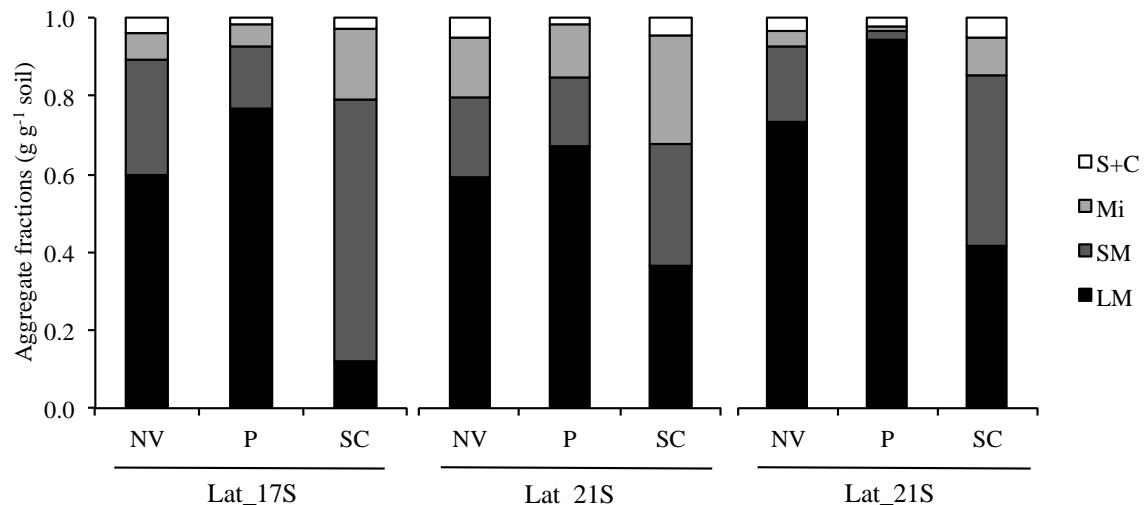


Figure 6 - Aggregate fractions at 0-30 cm soil depth for native vegetation (NV), pasture (P), and sugarcane (SC) soils from three locations in central-south Brazil. Aggregate fractions include large macroaggregates (LM; >2000 µm), small macroaggregates (SM; 250-2000 µm), microaggregates (Mi; 53-250 µm) and silt and clay (S+C; <53 µm)

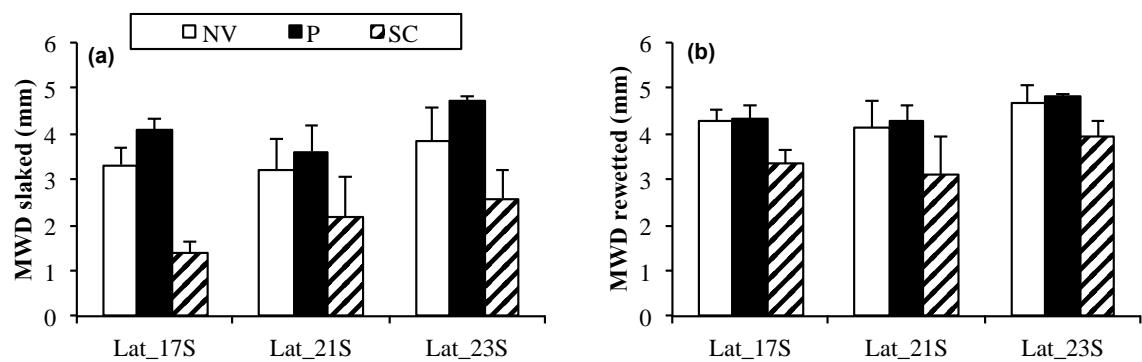


Figure 7 - Ratio of the mean weight diameter (MWD) of slaked and rewetted soils at 0-30 cm soil depth as a function of the land use change. Error bars denote standard deviation of the mean. Letters represent statistically significant differences between land uses at $p<0.05$

Table 5 - Mean weight diameter (MWD) for slaked and rewetted soil, and the normalized stability index (NSI) at 0-10, 10-20, and 20-30 cm soil depths under native vegetation (NV), pasture (P), and sugarcane crop (SC)

Depth	MWD Slaked			CV	MWD Rewetted			CV	NSI			CV
	NV	P	SC		NV	P	SC		NV	P	SC	
cm	----- mm -----	%	----- mm -----	%	----- mm -----	%	----- mm -----	%	----- mm -----	%	----- mm -----	%
<i>Lat_17S^a</i>												
0-10	3.71	4.03	1.37	9.41	4.43	4.30	3.44	6.61	0.86	0.87	0.57	25.48
10-20	3.23	4.07	1.29	12.36	4.22	4.33	3.11	8.32	0.79	0.87	0.58	22.47
20-30	3.03	4.15	1.46	10.12	4.21	4.39	3.43	6.47	0.77	0.83	0.53	22.98
<i>Lat_21S</i>												
0-10	4.33	3.82	2.11	10.19	4.64	4.47	2.81	16.63	0.82	0.73	0.80	16.11
10-20	2.71	3.84	2.66	26.38	4.40	4.45	3.30	10.95	0.72	0.93	0.63	24.98
20-30	2.51	3.19	2.05	33.99	3.32	3.96	3.23	26.05	0.61	0.79	0.70	24.02
<i>Lat_23S</i>												
0-10	4.36	4.74	2.93	12.79	4.62	4.79	4.18	3.53	0.95	0.93	0.80	11.72
10-20	3.73	4.76	2.33	17.06	4.57	4.85	3.46	6.87	0.85	0.88	0.72	14.13
20-30	3.43	4.67	2.51	17.95	4.22	4.82	4.11	12.80	0.84	0.91	0.73	14.46

^a Lat_17S, Southwestern region of Goiás state (17°56'16"S, 51°38'31"W); Lat_21S, West region of São Paulo state (21°14'48"S, 50°47'04"W); Lat_23S, South region of São Paulo state (23°05'08"S, 49°37'52"W).

CV, coefficient of variation.

2.2.6 Normalized stability index

NSI was influenced by LUC, with highest values found in pasture soils and lowest values found in sugarcane soils at all field sites (Figure 8). NSI in 0-30 cm soil layer was significantly larger in PA soil than under NV (Figure 9). The conversion from PA to SC significantly decreased NSI in 0-30 cm soil layer (Figure 9), and this effect was consistent at all sites, including a rapid decline from 0.85 to 0.56 at Lat_17S (Figure 8). This destabilization of soil structure was consistently observed in all soil layers analysed at Lat_17S and Lat_23S (Table 5). NSI tended to be higher in the 0-10 cm soil layer compared to the subsurface layers in NV soils (Table 5).

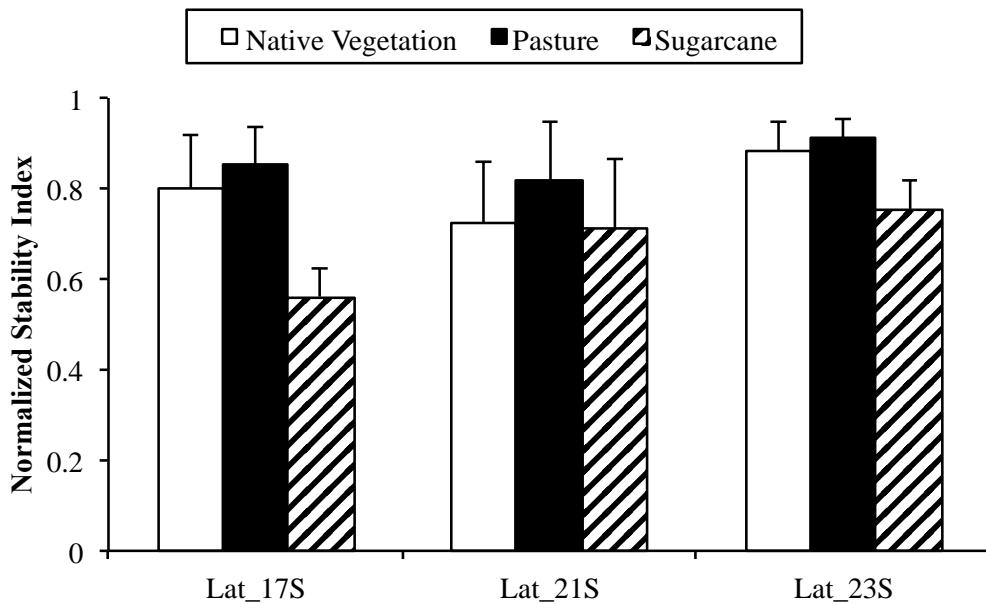


Figure 8 - Normalized stability index (NSI) of 0-30 cm soil layer in different land uses at three field sites (Lat_17S, Southwestern region of Goiás state ($17^{\circ}56'16''S$, $51^{\circ}38'31''W$); Lat_21S, West region of São Paulo state ($21^{\circ}14'48''S$, $50^{\circ}47'04''W$); Lat_23S, South region of São Paulo state ($23^{\circ}05'08''S$, $49^{\circ}37'52''W$). Error bars denote standard deviation of the mean

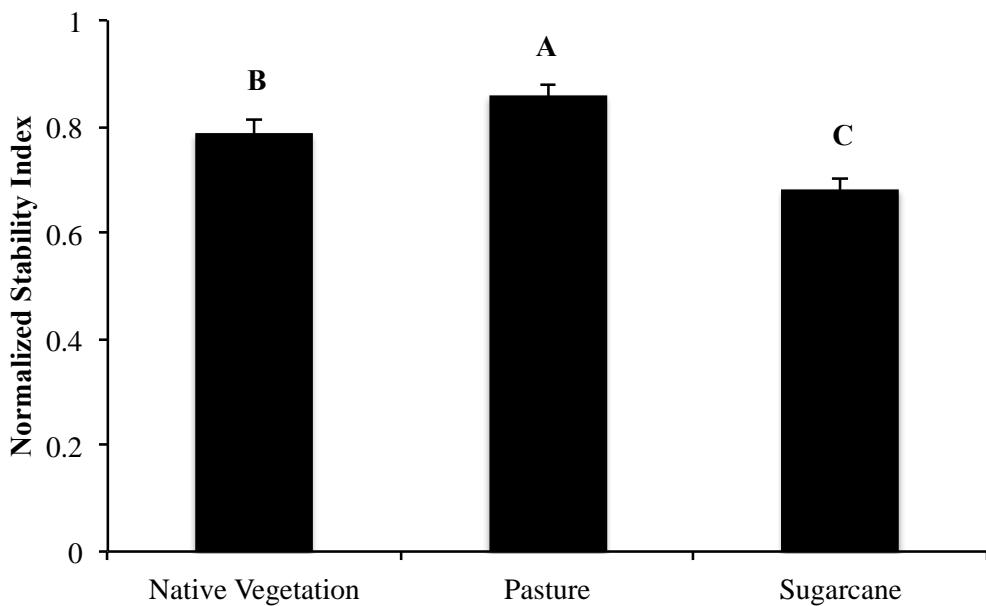


Figure 9 - Normalized stability index (NSI) of 0-30 cm soil layer in different land uses using a regional scale. Error bars denote standard error of the mean. Letters represent statistically significant differences between land uses according Scott-Knott test

2.2.7 Macroaggregate fractionation

Microaggregates within macroaggregates (53-250 µm) accounted for 74% of the macroaggregate weight in NV, and showed significant reduction to 67% in PA (Figure 10). Besides the reduction in microaggregates within macroaggregates, the silt + clay fraction (<53 µm) significantly increased from 9% of the macroaggregate weight in NV to 16% in PA (Figure 10). Such increase was observed at all field sites (Table 6).

From PA to SC, a significant increase in microaggregates within macroaggregates were observed (from 67% to 75% of the macroaggregate weight), and a significant decrease in particulate organic matter (POM) (>250 µm) was also found (Figure 10).

Table 6 - Proportion of macroaggregate weight found in the different fractions. Standard error of the mean is presented in parenthesis

Aggregate fraction	Native Vegetation	Pasture	Sugarcane
<i>Lat_17S^a</i> ----- % -----			
< 53 µm	4.82 (0.48)	7.17 (1.38)	13.61 (1.67)
53-250 µm	71.63 (1.59)	58.74 (2.18)	60.91 (1.42)
>250 µm	23.56 (1.46)	34.10 (1.83)	25.48 (1.32)
<i>Lat_21S</i>			
< 53 µm	9.94 (1.16)	13.46 (1.79)	7.38 (0.64)
53-250 µm	85.08 (1.74)	85.97 (1.59)	91.82 (0.73)
>250 µm	4.97 (0.86)	0.57 (0.33)	0.80 (0.17)
<i>Lat_23S</i>			
< 53 µm	13.26 (1.43)	26.59 (1.45)	20.75 (1.39)
53-250 µm	66.59 (3.78)	57.53 (4.97)	71.95 (1.48)
>250 µm	20.15 (4.27)	15.88 (4.25)	7.30 (0.88)

^aLat_17S, Southwestern region of Goiás state (17°56'16"S, 51°38'31"W);

Lat_21S, West region of São Paulo state (21°14'48"S, 50°47'04"W);

Lat_23S, South region of São Paulo state (23°05'08"S, 49°37'52"W).

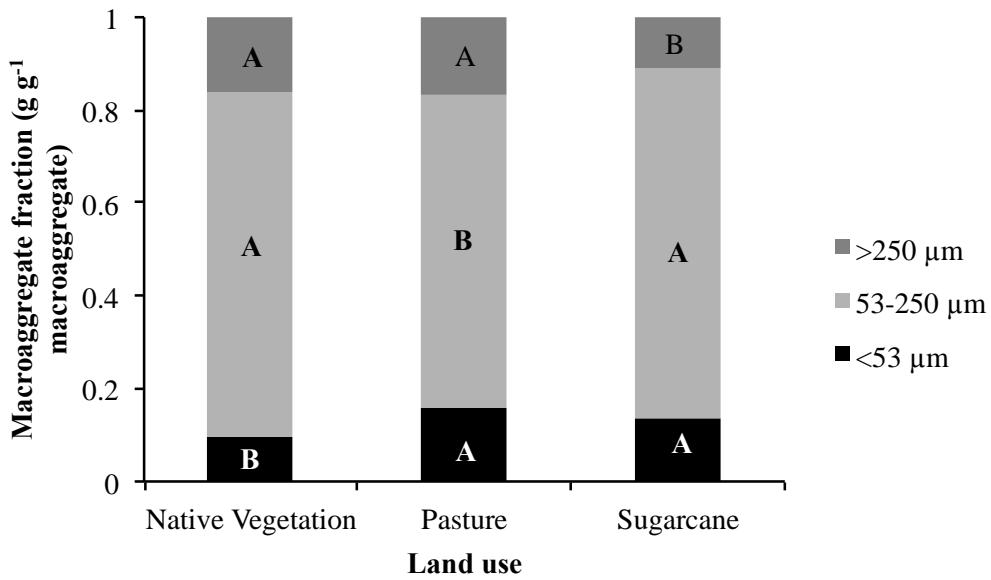


Figure 10 - Proportion of macroaggregate weight found in the different fractions, using a regional scale. Letters represent statistically significant differences between land uses according Scott-Knott test

2.2.8 Macroaggregate-associated C

The C stock found in the macroaggregates ($>2000 \mu\text{m}$ plus $250-2000 \mu\text{m}$) followed the same tendency to decline across LUC (Figure 11) as found in the whole soil's C stock (Figure 3). In contrast, the proportion of the total soil C stock found in macroaggregates increased from 72% in NV to 92% in PA to 95% in SC (Figure 11). Over 90.9% of the difference in total C stock between PA and SC could be accounted for by macroaggregate-associated C. More specifically, C associated with the microaggregates within macroaggregates accounted for an average of 60.1% of the difference in total SOC from PA to SC.

The C concentration of the mineral fraction ($<53 \mu\text{m}$) in the 0-30 cm soil trended to decline in the order: NV>PA>SC (Table 7).

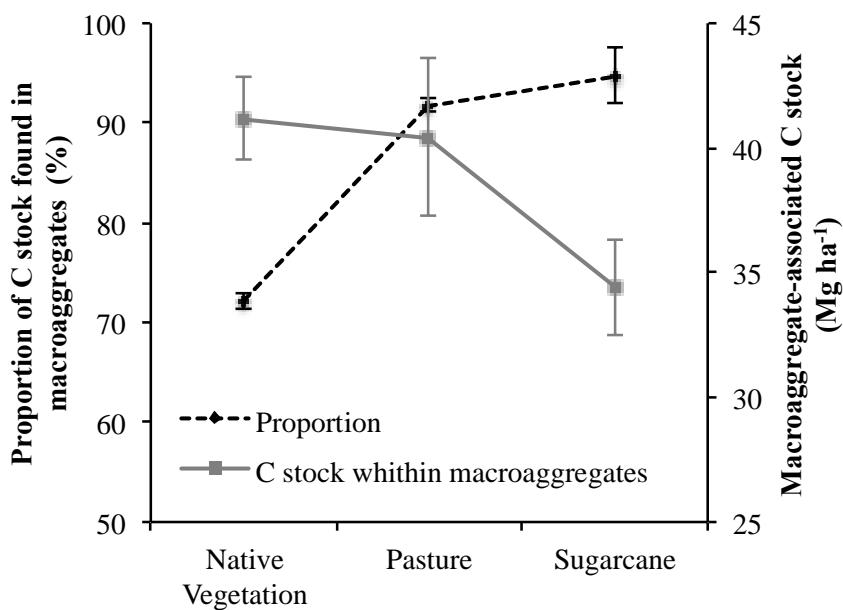


Figure 11 - Macroaggregate-associated C stock and the proportion of the total C stock found in macroaggregates in different land uses. Means of three chronosequences in different locations at Central-Southern Brazil. Error bars denote standard error of the mean

Table 7 - Carbon concentration of the macroaggregate fractions in 0-30 cm soil layer under different land uses. Coeficient of variation (%) is presented in parenthesis

Aggregate fraction	Native Vegetation	Pasture	Sugarcane
<i>Lat_17S^a</i> ----- g C kg ⁻¹ macroaggregate -----			
< 53 µm	75.78 (16.5)	53.64 (15.3)	30.88 (13.5)
53-250 µm	13.51 (11.9)	8.63 (10.7)	10.83 (8.9)
>250 µm	8.50 (22.7)	1.78 (5.4)	3.65 (24.4)
<i>Lat_21S</i>			
< 53 µm	41.78 (40.9)	23.83 (24.5)	30.48 (14.6)
53-250 µm	12.89 (60.3)	8.38 (20.3)	8.72 (18.3)
>250 µm	29.53 (58.5)	75.45 (12.6)	46.51 (55.5)
<i>Lat_23S</i>			
< 53 µm	30.76 (39.0)	28.65 (50.7)	21.51 (8.8)
53-250 µm	23.47 (33.4)	21.45 (44.6)	17.66 (11.0)
>250 µm	27.38 (24.8)	22.96 (43.4)	11.45 (12.3)

^a Lat_17S, Southwestern region of Goiás state (17°56'16"S, 51°38'31"W);

Lat_21S, West region of São Paulo state (21°14'48"S, 50°47'04"W);

Lat_23S, South region of São Paulo state (23°05'08"S, 49°37'52"W).

2.2.9 Macroaggregate turnover

The ratio of intra microaggregate-within-macroaggregate C to inter microaggregate-within-macroaggregate significantly increased from 1.3 in NV to 2.4 in PA, and decreased from 2.4 to 1.8 in SC (Figure 12). This result suggests that macroaggregate turnover, i.e. the rate of macroaggregate formation and degradation, was slower in PA compared to NV and SC.

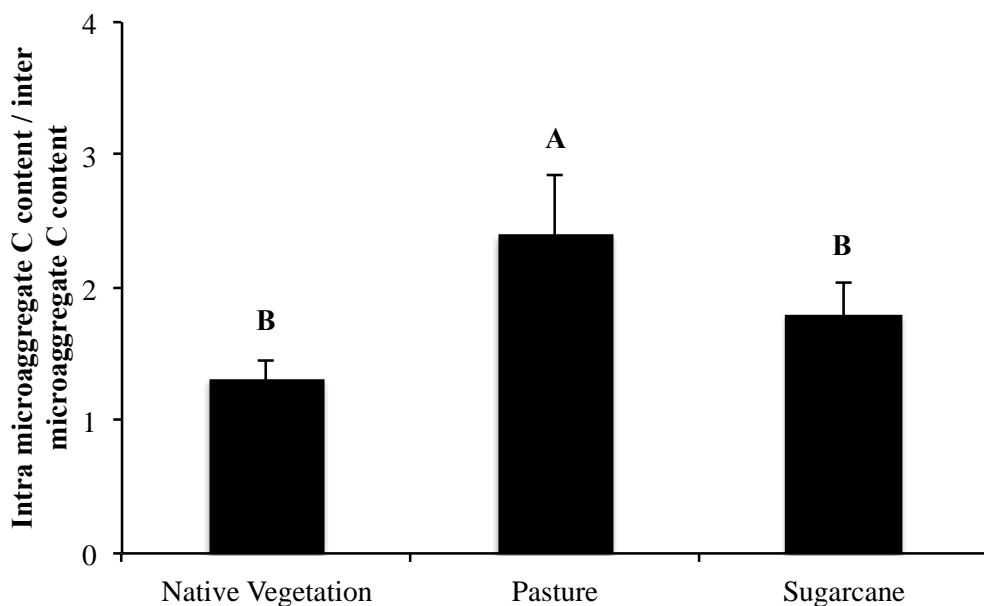


Figure 12 – Ratio of intra microaggregate-within-macroaggregate C content over inter microaggregate-within-microaggregate C content in the 0-30 cm soil layer of different land uses. Means of three chronosequence sites ($n=27$). Error bars denote standard error of the mean. Letters represent statistically significant differences between land uses according Scott-Knott test

In addition, the SOM humification index based on laser-induced fluorescence spectroscopy (H_{FIL}) of the microaggregates contained within the macroaggregates showed similar responses to LUC compared to the ratio presented in Figure 12, with a significant increase in SOM humification degree from NV to PA, and a significant decline from PA to SC (Figure 13).

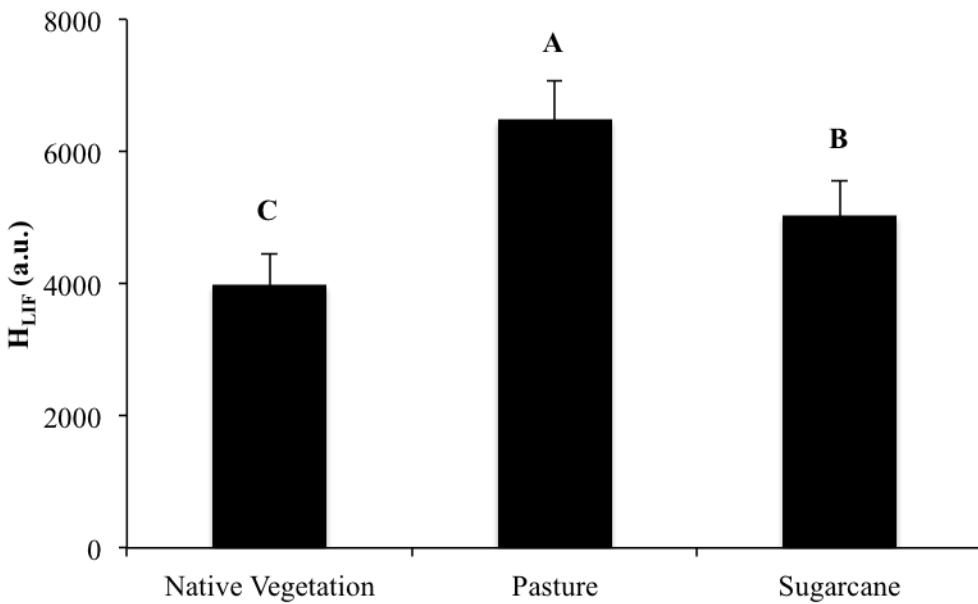


Figure 13 - Humification index of the organic matter occluded in microaggregates-within-macroaggregates (H_{LIF}) obtained through laser-induced fluorescence spectroscopy as affect by land use in the 0-30 cm soil layer. The H_{LIF} is expressed as arbitrary units (a.u.). Means of three chronosequence sites ($n=27$). Error bars denote standard error of the mean. Letters represent statistically significant differences between land uses according Scott-Knott test

2.2.10 Macrofauna abundance, taxa richness, Shannon index and evenness

The overall soil macrofauna density across all field sites ranged from 411 ± 70 (S.E.) individuals m^{-2} in the NV to $1,111 \pm 202$ individuals m^{-2} in the PA and 106 ± 24 individuals m^{-2} in the SC soils. Nonsignificant increases were found in the mean density from NV to PA, and sharp decreases occurred from pasture to SC in all sites (Figure 14a). The reduction in the size of the total community from pasture to SC amounted to 98% at Lat_17S, 78% at Lat_21S, and 90% at Lat_23S (Figure 14a).

A total of 14 soil macrofauna taxa were identified. All the 14 taxa occurred at NV, while 11 occurred at pasture, and 9 at SC soils. The NV at Lat_21S was the individual site with the largest mean number of taxa, 6.4 ± 0.7 , and the SC at Lat_17S had the lowest mean, 1.7 ± 0.2 (Table 8). The overall reduction in the taxonomic richness amounted to 24% from NV to PA, and 39% from pasture to SC, i.e. a mean loss of 53% of the taxonomic richness with the complete land use sequence. Lat_17S was the only site to show an increase in the mean number of taxa from NV (2.6 ± 0.4) to pasture (3.4 ± 0.4), although this was nonsignificant (Figure 14b).

Diptera, Blattodea, Hemiptera and Gastropoda were groups exclusively found under NV soils at all sites. More than 90% of the termites occurred in pasture soils, and the groups Oligochaeta, Dermaptera and Coleoptera were preferably found under pasture as well. Diplopoda was the single group with the majority of its individuals found in SC soils (Table 8).

Termites and ants were the most abundant taxa, comprising 43% and 21% of individuals identified, respectively. Termites comprised an average of 23% of the individuals at NV, 58% of the individuals at PA, and only 1% in SC soils. Ants accounted for an average of 26% of the individuals under NV, 5% under pasture, and 18% under SC. Coleoptera comprised 14% of the individuals identified, and its proportion on the communities increased with the complete land use sequence (13% of the individuals under NV, 17% under PA, and 21% in SC soils). Earthworms accounted for 10% of the total individuals, and were proportionally more abundant under anthropogenic land uses (17% of the individuals under pasture and 12% of the individuals under SC) than under NV (3%) (Table 8).

At all field sites there were significant effects of LUC on diversity (H') (Figure 14c). The overall H' dropped from 1.6 ± 0.2 in the NV to 1.0 ± 0.2 in the PA, and to 0.7 ± 0.2 in the SC soils (Figure 15). Although there were no statistical differences on the taxa distribution (E) among the land uses, there was evidence of a tendency for one or a few taxa to dominate the community with LUC (Figure 14d). The smallest value for E (0.3 ± 0.1) was found under pasture at Lat_17S.

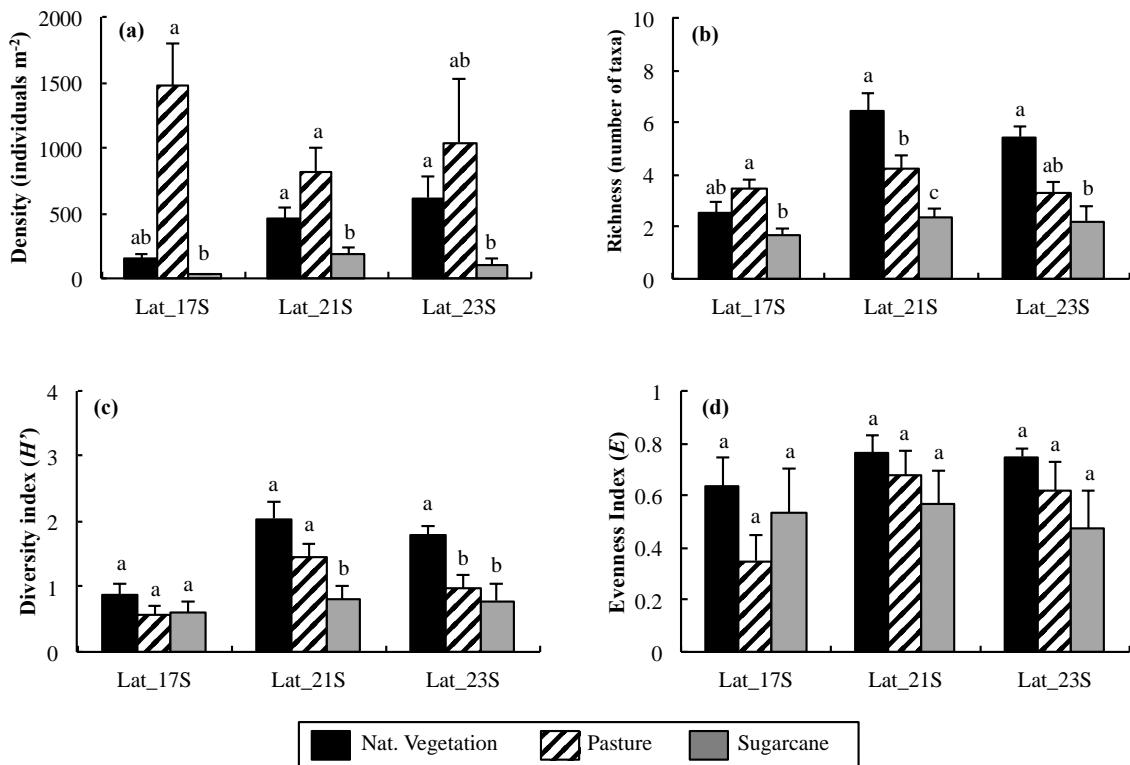


Figure 14 - Soil macrofauna density (a), taxonomic richness (b), diversity (c), and taxa distribution (d) in the 0-30 cm soil layer as a function of the land use change. $n = 9$, error bars denote standard error of the mean. Within field sites, means followed by the same letter are not statistically different ($p < 0.05$)

Table 8 - Mean density (individuals m⁻²) and standard error of soil macrofuna groups in the 0-30 cm layer. n = 9

Taxa	Common name	Taxonomic level	Lat_17S			Lat_21S			Lat_23S		
			NV	PA	SC	NV	PA	SC	NV	PA	SC
<i>Earthworms</i>											
Oligochaeta	Earthworms	Subclass	4 (2.4)	9 (5.4)	4 (2.4)	21 (9.2)	373 (102.9)	25 (7.1)	9 (3.9)	60 (15.5)	11 (7.1)
<i>Insects</i>											
Isoptera	Termites	Order	66 (26.7)	1093 (309.2)	0	11 (7.1)	78 (66.4)	0	151 (72.4)	924 (488.5)	2 (1.8)
Hymenoptera	Oth. than ants	Order	9 (3.9)	0	4 (2.4)	7 (3.9)	2 (1.8)	2 (1.8)	0	0	0
Formicidae	Ants	Family	4 (2.4)	55 (21.5)	0	144 (70.0)	78 (37.4)	2 (1.8)	272 (102.9)	14 (5.6)	55 (30.4)
Coleoptera	Beetles	Order	25 (10.7)	308 (277.7)	11 (4.6)	82 (30.5)	247 (64.2)	21 (13.6)	25 (10.4)	5 (2.7)	20 (10.6)
Blattodea	Cockroaches	Order	0	0	0	0	0	0	2 (1.8)	0	0
Dermoptera	Earwigs	Order	0	0	2 (1.8)	11 (6.0)	16 (7.1)	2 (1.8)	0	0	0
Diptera	Mosquitoes	Order	0	0	0	12 (7.4)	0	0	5 (5.3)	0	0
Hemiptera	Bugs	Order	0	0	0	2 (1.8)	0	0	0	0	0
<i>Myriapods</i>											
Diplopoda	Millipedes	Class	4 (2.4)	0	14 (4.2)	44 (25.8)	0	92 (38)	32 (6.0)	30 (12.9)	7 (4.7)
Chilopoda	Centipedes	Class	2 (1.8)	7 (2.8)	0	16 (8.4)	9 (6.0)	36 (35.6)	82 (24.2)	5 (2.7)	9 (4.7)
<i>Arachnids</i>											
Araneae	Spiders	Order	0	2 (1.8)	0	25 (7.6)	2 (1.8)	0	30 (11.8)	2 (1.8)	2 (1.8)
Scorpiones	Scorpions	Order	0	2 (1.8)	0	0	0	0	5 (5.3)	0	0
<i>Molluscs</i>											
Gastropoda	Snails	Class	2 (1.8)	0	0	4 (2.4)	0	0	5 (3.8)	0	0
<i>Crustaceans</i>											
Isopoda	Woodlouse	Order	37 (37.3)	0	0	84 (64.4)	9 (7.1)	0	0	0	0

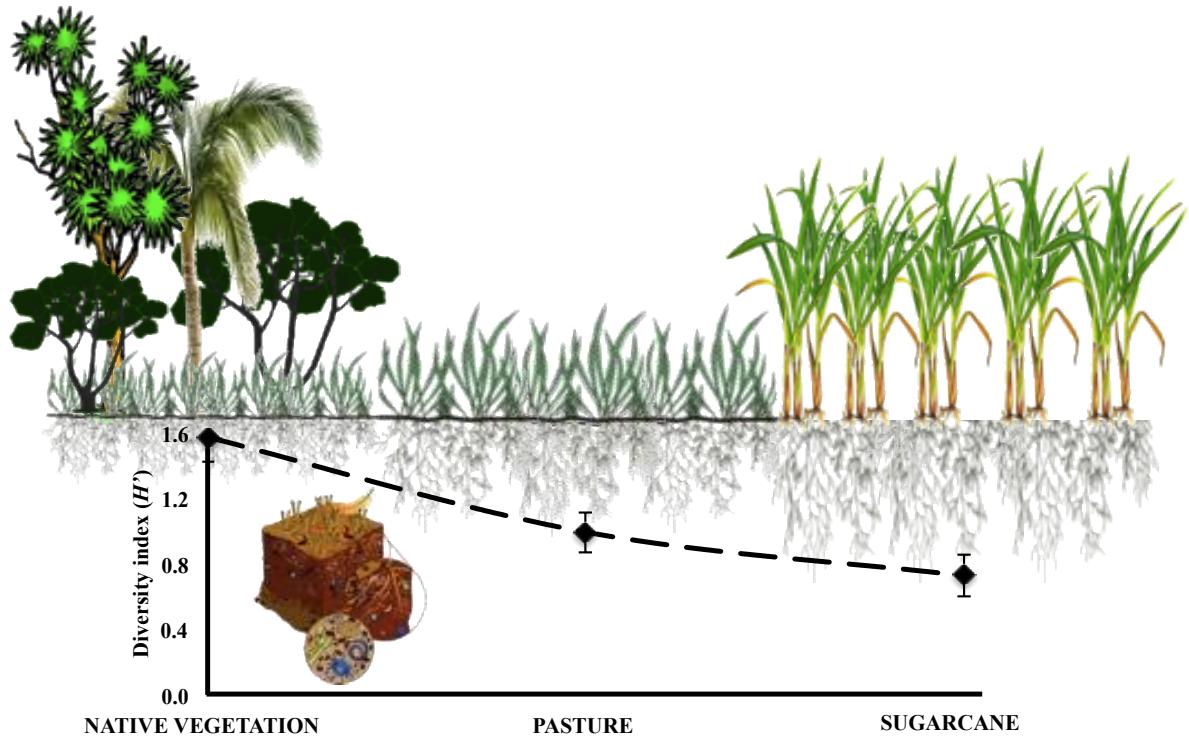


Figure 15 - Overall mean of soil macrofauna Shannon index in the 0-30 cm soil layer. $n = 27$, error bars denote standard error of the mean

2.2.11 Macrofauna vertical distribution and functional groups

The top layer 0-10 cm of the soil pedon harbored 69% of the organisms. Under SC an average of 87% of the organisms were found in the 0-10 cm layer, while this value was 80% in NV and 63% in PA. Despite substantially altering the abundance and taxonomic richness of the macrofauna community, LUC did not change the vertical distribution based on the proportion of taxa found in the different soil layers; there was a rate of 2:1 between the number of taxa in the 0-10 cm layer and the number of taxa in both 10-20 cm and 20-30 cm layers at all land uses (Figure 16).

The land use sequence had significant effects on trophic levels. Generalist predators were the more abundant functional group under NV, followed by the generalist detritivores. Detritivores were the more abundant group in pasture soils. In SC soils a similar number of detritivores and predators were found. Herbivore was the least abundant group, and their densities were found to be 4.1 ± 2.4 under NV, 5.3 ± 2.7 in pasture soils, and 1.2 ± 0.8 under SC. The detritivores reached 164.1 ± 36.2 individuals m^{-2} under NV, 859.3 ± 198.0 under pasture, and dropped to 51.6 ± 15.2 under SC. Similar densities of predators were found in NV and pasture soils (242.4 ± 56.5 and 245.9 ± 97.7 individuals m^{-2} , respectively), while this group diminished to 53.3 ± 20.0 individuals m^{-2} under SC (Figure 17).

The PCA shows the distribution of the land uses NV, PA and SC, using the functional groups (herbivore, detritivore and predator) as explanatory variables (Figure 18). The first axis explained 48.1% of the variance, while the second axis explained 35.9%. The NV areas were associated with the abundance of herbivorous and detritivores, while pasture sites were mainly associated with the herbivorous and predators. Sugarcane soils were oppositely grouped to the abundance of all functional groups.

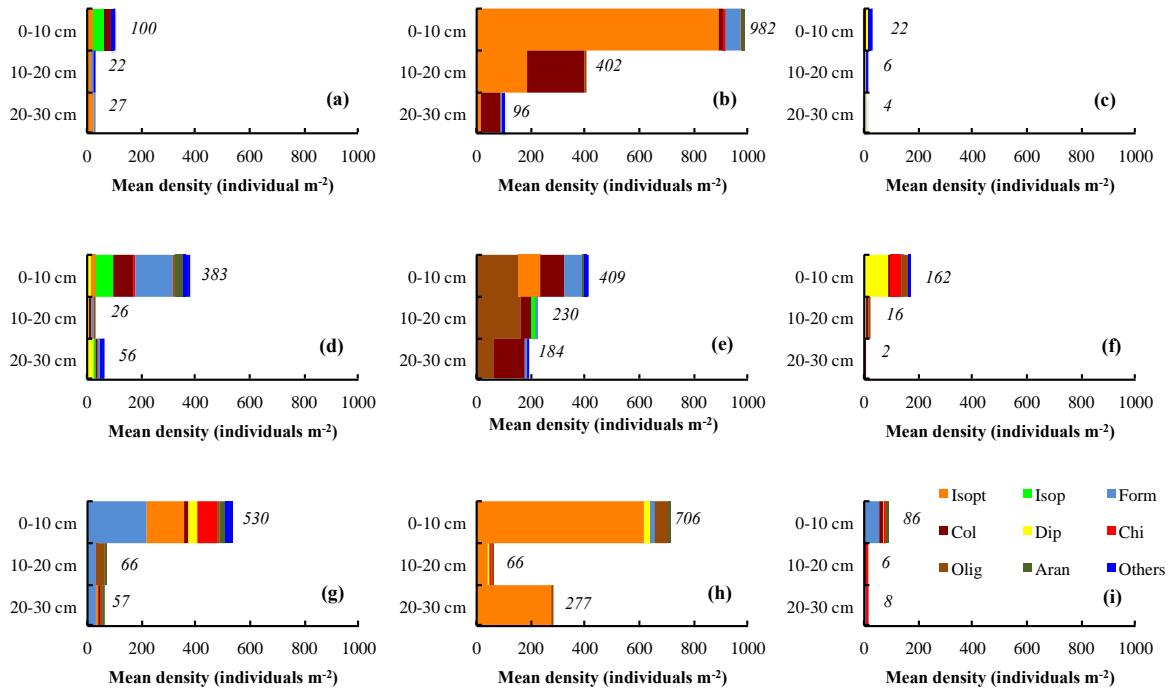


Figure 16 - Vertical distribution and taxonomic group composition of soil macrofauna within soil pedon under native vegetation, pasture, and sugarcane at Lat_17S (a, b & c, respectively), at Lat_21S (d, e & f, respectively), and at Lat_23S (g, h & i, respectively). Numbers in italics refer to total density in each layer. $n = 9$

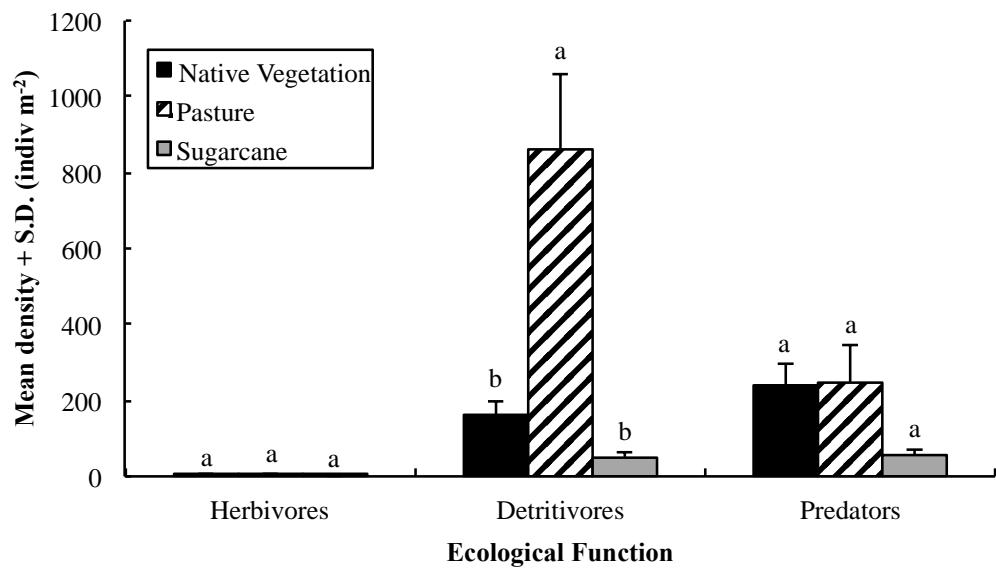


Figure 17 - Overall mean of the abundance of soil macrofauna functional groups in the 0-30 cm soil layer as a function of the land use change. $n = 27$. error bars denote standard error of the mean. Within functional groups, means followed by the same letter are not statistically different ($p < 0.05$)

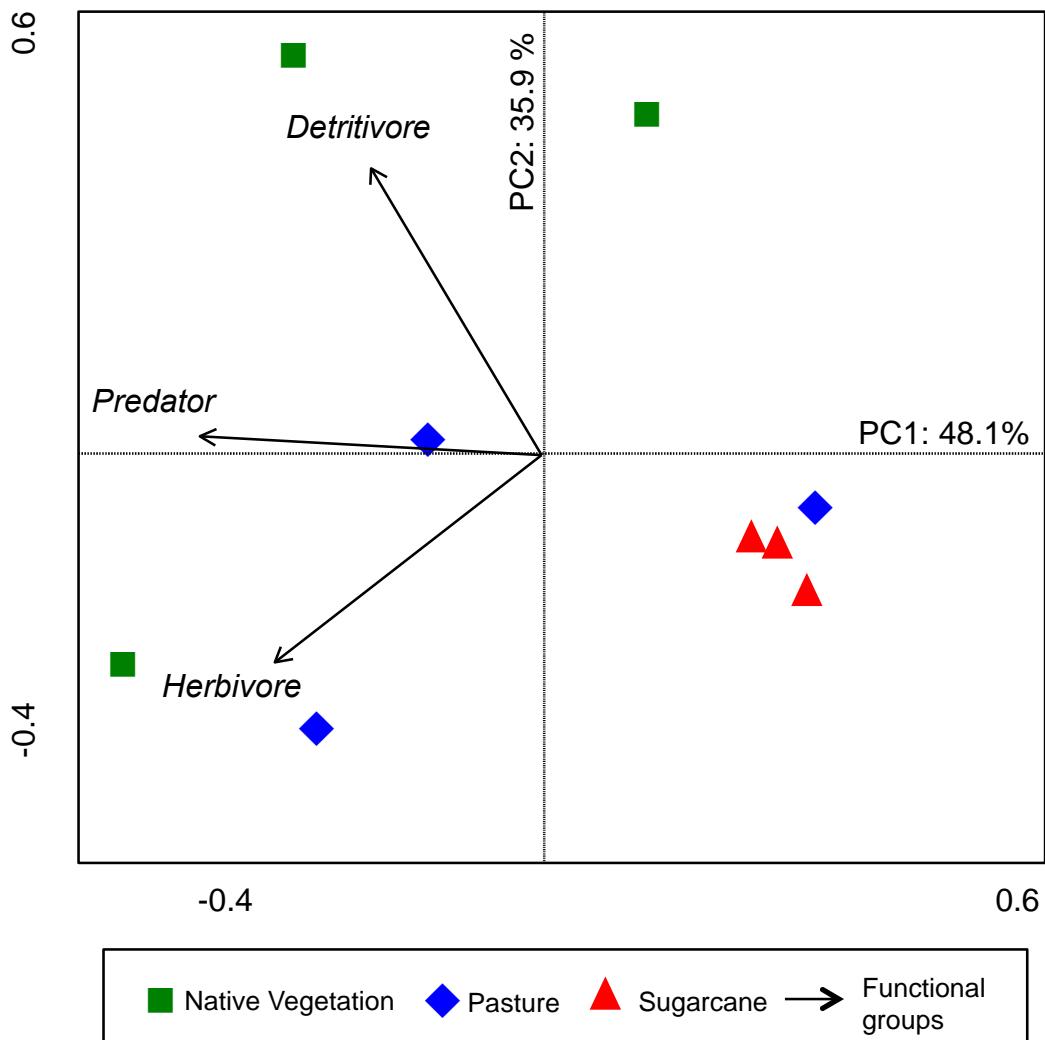


Figure 18 - Principal component analysis of the abundance of macrofauna functional groups found under native vegetation, pasture, and sugarcane

2.2.12 Macrofauna, soil structural stability and C allocation

Strong correlations were found between soil structure and macrofauna variables, while the contents of clay and sand in the soil did not present direct correlations with soil structure (Figure 19a). The first axis of the PCA explained 46.4% of the variance and the second axis 26.2%. The density of macroinvertebrates was positively and strongly associated with both large macroaggregates and structural stability (NSI), and negatively related to small macroaggregates, microaggregates and silt + clay. Functional diversity of soil macrofauna as well as taxonomic richness was not closely associated with any soil structure variables. The functional group with the strongest correlation with soil structure stability was the detritivore group, showing strong and positive correlations with large macroaggregates and NSI, and negative correlations with all fractions lower than 2000 µm. Pasture sites were associated with

higher macrofauna densities, taxonomic richness, detritivorous densities, and also higher large macroaggregates and structural stability. Sugarcane sites, on the other hand, were opposed to these variables, and linked to higher proportions of small macroaggregates, microaggregates, and disperse silt + clay particles (Figure 19a).

The PCA in Figure 19b shows the relationship between soil structure variables and the densities of detritivore taxa, besides the distribution of the field sites. The first axis of the PCA explained 37% of the variance and the second axis 25.8%. High proportions of large macroaggregates and higher NSI were mainly related to high densities of isoptera and oligochaeta, and pasture sites were grouped near these variables, while sugarcane sites were opposed.

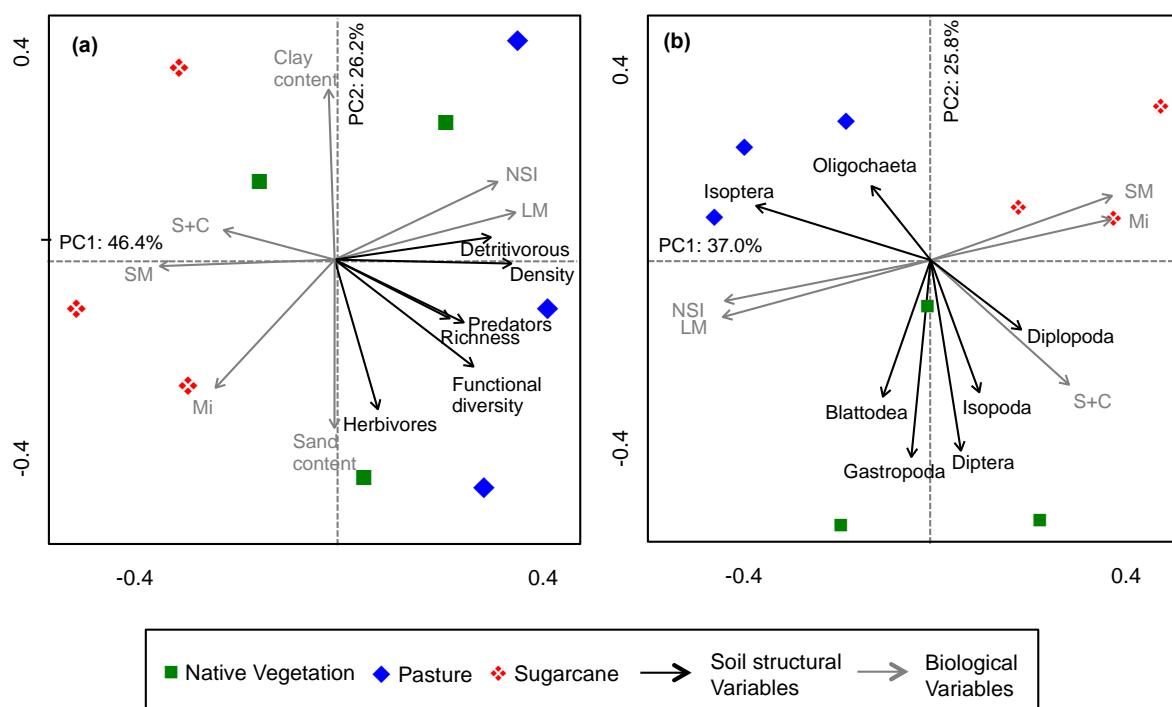


Figure 19 - Principal component analysis of soil structure variables NSI (normalized stability index) and soil aggregates size fractions: S+C (silt plus clay; <53 µm), Mi (microaggregates; 53 – 250 µm), SM (small macroaggregates; 250 – 2000 µm), LM (large macroaggregates; 2000 µm), using soil macrofaunal attributes as explanatory variables: (a) Density (macroinvertebrates individuals m⁻²), Richness (taxonomic richness: number of taxa), and the density of the functional groups Herbivorous, Detritivorous and Predators; (b) Detritivorous taxa

There was a strong and positive correlation ($R^2=0.68$, $P=0.006$) between the density of earthworms and the inter microaggregate POM C (Figure 20).

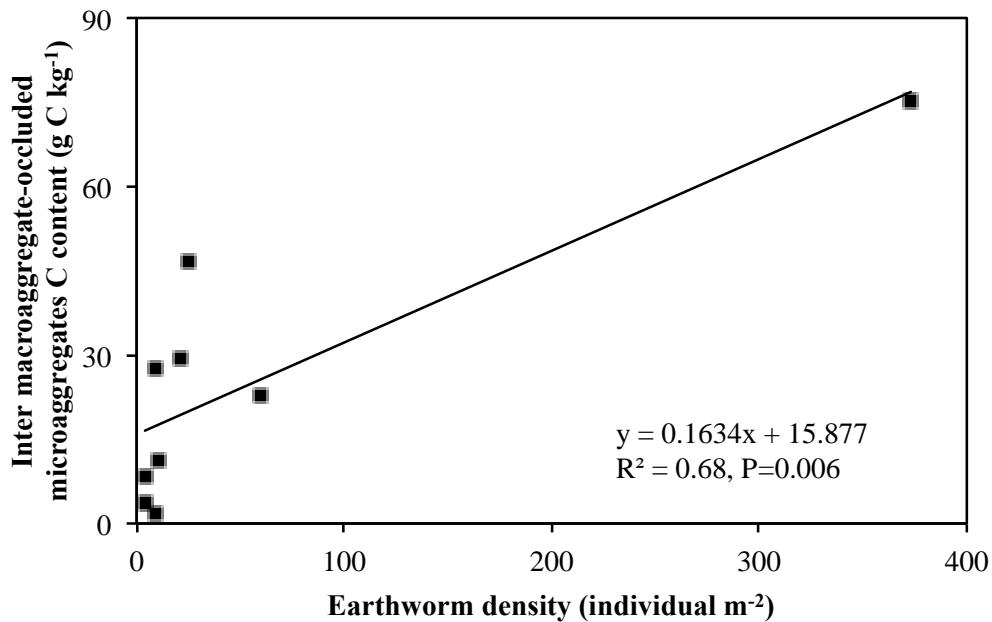


Figure 20 - Relationship between the earthworm density and the C concentration in the inter microaggregates particulate organic matter occluded within macroaggregates

2.3 Discussion

2.3.1 Land use intensity as a driver of changes in SOM concentration and stock

In both land use transitions evaluated here changes in soil C and N were always correlated, i.e. where C was gained, N was also gained, and where C was lost, N was also lost. These results are in agreement to those reported by Murty et al. (2002) in a review of a large body of the literature on changes in soil C and N following the conversion of forests to pasture and cultivated land. At Lat_23S the baseline for C and N levels is greater than at Lat_17S and Lat_21S, and it is clearly due to the higher clay content at Lat_23S, with a mean of 633 g kg^{-1} of clay (Table 1). Soil C is linearly related to clay content because clay particles in soil provide a reactive surface area for the stabilization of C in organomineral forms, and these particles tend to form aggregates which physically protect C from decomposition (SCHIMEL et al., 1994).

The SOM depletion after the conversion of natural ecosystems to pastures observed here are consistent with several studies in tropical areas (ELMORE; ASNER, 2006;

GARCIA-OLIVA et al., 2006; MAIA et al., 2009; COSTA et al., 2011). The typical pasture management in tropical systems is typified by the pasture degradation and reduced productivity for grazing animals, due to high weed infestation, bare soil and soil erosion, in agreement with our observations for these sites (MAIA et al., 2009). This management has been the driver of SOM losses after land conversion from forests to pastures (FEARNSIDE; BARBOSA, 1998; MURTY et al., 2002; CERRI et al., 2007; MAIA et al., 2009). There are few studies assessing the effects of sugarcane expansion into pastures on SOM levels. Rossi et al. (2013) reported SOM losses in the upper 30 cm soil after conversion from pasture to sugarcane in Brazilian Cerrado. The effect of time since land use conversion from pasture to sugarcane on SOM depletion can be observed by contrasting the oldest and the youngest sugarcane sites - 36% less organic C was found under sugarcane cropped for 23 years compared to pasture at Lat_23S, while only 4% less organic C was found under sugarcane cropped for 3 years at Lat_21S (Table 2).

Increases in SOM ^{15}N enrichment have been described as a result of the progress in the mineralization, nitrification, denitrification and volatilization processes (HOGBERG, 1997; BUSTAMANTE et al., 2004), and are typically accompanied by reductions in SOM levels (HOGBERG, 1997; MENDONÇA et al., 2010), indicating organic matter decomposition. This inverse relationship between SOM and $\delta^{15}\text{N}$ levels was found here: (i) in deeper soil layers at all field sites and land uses, with higher values of $\delta^{15}\text{N}$ in deeper layers (Table 3), indicating a more humified SOM with soil depth (KRULL; BESTLAND; GATES, 2002; KRAMER et al., 2003), and (ii) in the land use transition from PA to SC, where the average C levels decreased from 15.5 to 12.7 g kg $^{-1}$ (Table 2), and the average $\delta^{15}\text{N}$ values increased from 7.2‰ to 8.0‰ (Table 3). These latter results are in line with findings of Rossi et al. (2013), indicating more intensive decomposition of humic substances by microorganisms and a possible accumulation of more recalcitrant SOM under sugarcane. The conversion of NV to PA, however, trended to decrease both SOM and $\delta^{15}\text{N}$ values (Table 3). These finds added to significant SOM decreases from NV to PA in 0-10 cm layer (Table 2) suggest a pasture priming effect on original SOM decomposition in the surface layer. In contrast to the other field sites, at Lat_17S pasture soil was mostly enriched in ^{15}N relative to the NV, and it is likely a result of the typical low $\delta^{15}\text{N}$ value in soil under Cerrado vegetation, promoted by the high content of legume species in these areas (BUSTAMANTE et al., 2004; COSTA JUNIOR et al., 2011; ROSSI et al., 2013). The $\delta^{15}\text{N}$ values found at Lat_17S were similar to those reported by Costa Junior et al. (2011) and Rossi et al. (2013) in the same region.

Evaluations of land use system impacts on soil chemical attributes using the stocks approach removes the bias that can happen when samples are collected from soils under scenarios with different effects on soil bulk density (VARVEL; WILHELM, 2011).

The SOC stocks depletion from NV to PA observed in this study was consistent with other studies (ELMORE; ASNER, 2006; GARCIA-OLIVA et al., 2006; MAIA et al., 2009; ASSAD et al., 2013). Overall, SOC stocks decreased at an average rate of $0.4 \text{ Mg ha}^{-1} \text{ yr}^{-1}$ after the conversion of NV to PA (Figure 4a). This rate was greater than $0.28 \text{ Mg ha}^{-1} \text{ yr}^{-1}$ reported by Maia et al. (2009) for the conversion of native forests to pastures in Brazilian cerrado.

An average proportion of 63% C_{C4} for pasture soils was found (Figure 3), which is the same result found by Assad et al. (2013) in a study encompassing a broad range of climatic conditions and soil composition in Brazil. The “typical” pastures in Brazil have been suggested as a net carbon source (FEARNSIDE; BARBOSA, 1998; MAIA et al., 2009). Improved management to promote high grass production results in greater inputs of grass-derived C to pasture soils and larger C stocks compared to extensive pastures such as those utilized in this study (NEILL et al., 1996; DON; SCHUMACHER; FREIBAUER, 2011). The maintenance of large SOC stocks in PA at Lat_23S (Figure 3c) is likely to be related to the clayey texture of the soil. Assad et al. (2013) found that the soil texture is a prominent key controller of SOC stocks in Brazilian pastures, and suggested that sandy soils should be avoided in order to implement grasslands that are able to maintain at least the same SOC stock compared to the native vegetation. In addition, the low grazing pressure management at this site (Table 1) has also contributed to maintain similar SOC stocks under PA related to NV, once higher grazing pressure have been reported to decrease SOC stocks in tropical grasslands (PRINGLE et al., 2014).

The conversion of NV to PA also decreased N stocks consistently at all sites (Figure 5a), even at Lat_21S where there were annual inputs of N fertilizers in PA (Table 1). Pringle et al. (2014) reported a significant loss of total nitrogen from the topsoil of a tropical grassland as a result of animal grazing. It can be attributed to the N losses by N harvest in grazing, NH₃ volatilization, desnitification, and leaching (HAYNES; WILLIAMS, 1993; ASSMANN et al., 2014).

The absence of frequent tillage on pasture land is likely to explain part of the frequently observed higher SOC stocks in pastures compared to sugarcane crops in Brazil (EGESKOG et al., 2014; MELLO et al., 2014). Every five years a cultivation cycle is carried in sugarcane fields with ploughing and fertilization for planting of new stem cuttings,

reducing soil carbon stocks over time (MELLO et al., 2014). Sugarcane soils had similar or larger SOC stocks than pastures where this land use transition had less than 5 years (Figure 3a,b), while SOC stock losses of 29.1 Mg ha^{-1} were observed at Lat_23S with more than 20 years of sugarcane crop (Figure 3c), including more than 10 years under burned harvest management, which depletes SOC stocks over time (GALDOS; CERRI; CERRI, 2009). The period of burned harvest management is likely to have produced less enriched ^{13}C signal (PINHEIRO et al., 2010; RACHID et al., 2012). Mello et al. (2014) reported a SOC stock loss of 20.7 Mg ha^{-1} and a reduction rate of $1.0 \text{ Mg ha}^{-1} \text{ yr}^{-1}$ for 0-30 cm soil layer after 20 years of conversion from pasture to sugarcane in Central-South Brazil.

At Lat_23S, more than 20 years of sugarcane crop caused a reduction of 1.9 Mg ha^{-1} in N stock compared to the pasture soil, even with high annual inputs of organic fertilizers in sugarcane at this site. The sugarcane burned harvest, where the dry leaves and tops are burning pre-harvest, practiced for more than 10 years after land transition in this site, potentially depleted nitrogen levels over time (ROBERTSON, 2003).

2.3.2 Contribution of the macroaggregate-associated C fraction to LUC-induced changes in soil carbon stocks

Over 90.9% of the difference in total C stock between PA and SC could be accounted for by macroaggregate-associated C. The increased contribution of the C stock found within macroaggregates to the total soil C stock across LUC (Figure 11) indicates that the more insensitive and hostile to the SOM the land use is, the more important this fraction becomes as a carbon stabilization site. More specifically, C associated with the microaggregates within macroaggregates accounted for an average of 60.1% of the difference in total SOC from PA to SC, and the contribution of this specific fraction to the total soil C stock also increased across LUC (from 53.6% in NV to 60.8% in PA to 68.6% in SC). This result provides further support for the preferential C stabilization inside the macroaggregate-occluded microaggregates (SIX; PAUSTIAN, 2014), and shows that the SOM stabilized within this fraction is resistant to change after LUC with increase in land use intensity.

Even with contrasting soil textures among the three field sites (Table 1), the effects of LUC on size distribution of soil aggregates were broadly consistent (Figure 6), which suggests lower control of soil texture on soil stability responses to LUC. Stable macroaggregates ($> 250 \mu\text{m}$) dominated soil aggregate distributions at all sites, including the coarse-textured soils at Lat_21S (Figure 6), indicating that soils were all highly aggregated

irrespective of the texture. High levels of aggregation have often been reported for tropical weathered soils (ZOTARELLI et al., 2005; BARTHES et al., 2008).

The conversion of NV to pasture increased the proportion of the large macroaggregate class ($> 2000 \mu\text{m}$), and the proportion of thinner aggregate units correspondently decreased. This improved abundance of water-stable larger aggregates from NV to pasture was also evidenced by the higher NSI in pasture soils. Some studies have linked pastures with high structural stability in tropical soils (PINHEIRO; PEREIRA; ANJOS, 2004; SALTON et al., 2008; LAVELLE et al., 2014). The absence of frequent tillage on pasture lands and the root biomass of C4 tropical grasses provide ideal conditions for both macroaggregate formation and stabilization via biological activity (JASTROW, 1987; PINHEIRO; PEREIRA; ANJOS, 2004). In this study, pasture soils presented the highest contents of water-stable macroaggregates (Figure 6), highest structural stability (Figure 9), and the slowest macroaggregate turnover (Figure 12), which improve the preservation of low-humified POM through physical protection mechanisms and explains the tendency to decreased $\delta^{15}\text{N}$ values from NV to PA (Table 3). The values obtained for soil aggregate distribution and MWD under NV and pasture soils are similar to those reported by other studies in Brazilian soils (PINHEIRO; PEREIRA; ANJOS, 2004; SALTON et al., 2008).

On the contrary, the conversion of pastures to sugarcane decreased the proportion of large macroaggregates and correspondently increased all thinner aggregate units. This breakdown of the larger aggregates from pasture to sugarcane was also indicated by the lower NSI in sugarcane soils. Several studies have reported detrimental effects on soil structural stability when pastures are converted to cropland (e.g. SPOHN; GIANI, 2011; LIU et al., 2014). The depletion of structural stability after only 3 and 4 years of cultivation, as showed respectively at Lat_17S and Lat_21S, evidenced a short-term increase in erosion susceptibility after LUC from pasture to sugarcane. Silva et al. (2007) reported a reduction in aggregate stability after 2 years of sugarcane cultivation in Northeast Brazil. These authors also related a recovery of aggregate stability after 18 years of cultivation, and suggested that the roots of sugarcane provide great structuring power to improve aggregate stability over time. Our results at Lat_23S, however, showed that more than 20 years of sugarcane crop the reductions in large macroaggregates, MWD, and NSI remained evidenced. The C loss from PA to SC at Lat_23S was promoted by the respiration of C_{C4} due to detrimental effects of sugarcane crop practices on soil structural stability (Figure 8 and 9), which accelerate macroaggregate turnover (Figure 12) and the oxidation of occluded POM fixed during the pasture period. Marked reductions in macroaggregates and MWD in sugarcane soils compared to uncropped

soils were also reported by Blair (2000) in Australia. Every five years a cultivation cycle is carried in sugarcane fields with ploughing and fertilization for planting of new stem cuttings. These tillage operations destroy the habitats of soil animals that act as soil engineers. Moreover, the successive machine traffic, due to fertilization, weed control, harvest and transport operations, is one of the main causes of soil compaction (LOZANO et al., 2013; ABDOLLAHI et al., 2014), which is favored by the direct disruption of macroaggregates. It is worth mentioning that the green cane harvesting with trash retention have been applied at all three field sites, and this unburned harvesting system is known to increase SOM contents, as well as increase the concentrations of various labile organic matter fractions (GRAHAM; HAYNES; MEYER, 2002b). These changes in organic matter content and quality should improve soil aggregation (GRAHAM; HAYNES; MEYER, 2002a), which suggest that the reductions in structural stability described here could be even boosted if burning was practised prior to harvest.

Surprisingly, the larger macroaggregate turnover observed in PA soils (Figure 12) did not result in greater amounts of macroaggregate-occluded microaggregates (Figure 10) or higher concentration of C (Table 7) within this fraction. This finding is in agreement with those reported by Denef et al. (2007), and suggests that the C sequestration due to preferential C stabilization inside this fraction is rather caused by a greater stability and slower turnover of the macroaggregate-occluded microaggregates. Such assumption was strongly supported by the finding that the slower the macroaggregate turnover is, the more humified is the SOM associated with the macroaggregate-occluded microaggregates (Figure 13). Although more validation is needed, we suggest that H_{FIL} from macroaggregate-occluded microaggregate samples may be a direct measure of the macroaggregate turnover, removing the exhaustive and time-consuming step of SOM fractionation to obtain the ratio of fine intra POM to coarse inter POM largely used as a relative measure of the turnover of macroaggregates.

2.3.3 Role of soil macrofauna in aggregation and SOM storage

Contrary to annual crops, pastures cultivated from NV areas in Cerrado environment generally provide suitable soil habitats for a high abundance of soil macroinvertebrates (DECAENS et al., 2004). Our study confirms other studies that show the size of the macrofaunal community in tropical soils trending to increase over time after conversion of native vegetation to pasture (BLACK; OKWAKOL, 1997; BENITO et al., 2004; DECAENS et al., 2004; LAVELLE et al., 2014). Increased C inputs from large roots and aboveground biomass likely provided better quality food to the organisms (LAVELLE et al., 2014).

Average termite density increased ninefold while earthworm densities increased thirteenfold from NV to pasture, and these two groups largely dominated the community under pasture (Table 8). Consequently, there was a decrease in evenness (E) at all field sites from NV to pasture (Figure 14d), particularly at Lat_17S where termites accounted for 74% of the individuals under pasture (Table 8). Findings of Fonte et al. (2012) suggest that soil macrofauna can substantially enhance nutrient uptake and biomass production in tropical pastures, and that these interactions between tropical grasses and soil macrofauna enhance soil structural stability by creating the positive feedback of increasing accumulation of SOM from plant roots within aggregates.

In general, the conversion of pasture to SC negatively impacted the density, taxonomic richness and H' of the soil macrofaunal community (Figure 14a, 14b and 14c). Sugarcane soils as well as the majority of croplands are managed at moderate to strong intensity, with deep tillage and plowing, and systematic use of chemical inputs, such as pesticides and fertilizers (BELL; STIRLING; PANKHURST, 2007). Despite the proportionally low number of agricultural pests in these soils, pesticides are commonly used, with negative impacts on entire belowground community. In the last decade a shift from manual to mechanical harvesting in Brazil has caused the rapid proliferation and spread of certain sugarcane pests (e.g. root spittlebug, curculionid, sugarcane borer) (DINARDO-MIRANDA; FRACASSO, 2013). Consequently, the application of chemicals is increasing, as it is still the more efficient alternative of control against these pests. The intensive use of mineral fertilizers as observed in SC soils negatively affects the density, diversity and equability of soil macrofaunal community (GILLER et al., 1997; SILESHI; MAFONGOYA, 2006; ALVES et al., 2008).

The concentration of macroinvertebrates in the upper 10 cm of soil in this study (Figure 16) was higher than in other studies (BARROS et al., 2003; PAULI et al., 2011), and unlike these studies, it seems disconnected with the vertical variations in the contents of soil organic carbon (SOC) (Table 2). SC soils, for example, appeared to have SOC homogenization in the upper 30 cm soil (Table 2) as a result of tillage operations: nevertheless 87% of the organisms were found in the upper 10 cm of soil (Figure 16). The vertical distribution is likely a function of the litter concentration at surface and the lower bulk density in the 0-10 cm soil layer (Table 1).

In spite of the fact that no significant differences were found among the land uses for both herbivore and predator populations, the multivariate analysis performed (PCA) evidenced that LUC affected trophic levels. PCA showed clearly the preferential occurrence of the three functional groups in NV and pasture soils rather than SC soils (Figure 18). Less

detrivores, particularly termites and earthworms, were observed in SC than in NV and PA soils (Figure 17). Conversion of PA to SC as well as the SC crop management involves tillage that destroys the habitats of detritivore invertebrates. According Lavelle et al. (1997) and Brévault et al. (2007) (LAVELLE et al., 1997; BREVAULT et al., 2007), the detritivore functional group is especially important as these organisms act as catalysts in the decomposition of organic matter. The relatively high population densities of predatory invertebrates found in pasture soils may be the result of the abundant detritivore population in these soils (ROBERTSON; KETTLE; SIMPSON, 1994).

Our findings confirmed the hypothesis that soil structural stability decreases with LUC in sugarcane expansion areas irrespectively of the soil texture, but consistently related to reductions in the size and composition of soil macrofaunal community (Figure 19a). It suggests that the inclusion of macrofauna parameters in structural equation models such as proposed by Chaudhary et al. (2009) and Barto et al. (2010) to examine the relative influences of biotic and abiotic factors to soil aggregation should minimize the high amount of unexplained variation in soil structure variables exhibited in those models (65% in CHAUDHARY et al., 2009 and 57-85% in BARTO et al., 2010). Moreover, the common sense that abiotic factors related to soil texture and clay mineralogy play a more important role in determining soil stability than does biotic factors in highly weathered surfaces mostly stems from studies in which the single biotic factor considered is long-dead soil C (SOM) (OADES, 1993; BARTHES et al., 2008; PENG et al., 2015), while live biota are likely to be more important for aggregate stability than SOM (BARTO et al., 2010).

The density of soil macrofauna was positively correlated with both large macroaggregates and soil structural stability (NSI) (Figure 19a). Some soil macrofauna animals feed on litter and organic residues on the soil surface, and incorporate these organic matters in the soil profile within stable soil aggregates (BOTTINELLI et al., 2015). Specifically the density of the detritivore functional group was strongly correlated with structural stability of soils (Figure 19a). It is no wonder this functional group comprises the most important soil engineers - earthworms and termites (LAVELLE et al., 1997). The soil engineering concept essentially makes reference to the ability of these organisms to move through the soil and to build biogenic structures with specific physical, chemical and microbiological properties (JOUQUET et al., 2006). In this study, higher densities of macrofauna animals were found in pasture soils (Figure 14), where a wide dominance of termites in the macrofaunal community was evidenced. Termites are very vulnerable insects that protect their colonies by improving soil structural stability against water flux or intrusion

of soil invertebrate predators into nests (JOUQUET et al., 2006). As pointed out by Six et al. (2004), soil-feeding termites influence the structural stability of soils, especially the microstructure, either by passing soil material through their intestinal system and depositing it as fecal pellets or by mixing the soil with saliva using their mandibles. A pronounced increase in earthworm's density from 21 ± 9 individuals m^{-2} in NV to 373 ± 102 individuals m^{-2} in PA occurred at Lat_21S, and at this site soil structural stability had pronounced increases from NV to pasture (Figure 8). Several studies have shown the importance of earthworms in soil aggregation (KETTERINGS; BLAIR; MARINISSEN, 1997; BOSSUYT; SIX; HENDRIX, 2006; AYUKE et al., 2011). As referred to recent review of Blouin et al. (2013), positive effects of earthworms on the proportion of water-stable macroaggregates have been widely demonstrated. Earthworms ingest soil as they burrow and forage on SOM, thus promoting the formation of stable aggregates rich in carbon (AYUKE et al., 2011). These observations explain why pasture soils were grouped near the biological variables and associated with high structural stability (Figure 19a).

The strong positive correlation found between the density of earthworms and the inter microaggregate POM irrespectively of the land use (Figure 20) provides further support to the direct involvement of earthworms in providing protection of POM in macroaggregates leading to a possible long-term stabilization of soil C (BOSSUYT; SIX; HENDRIX, 2005).

3 CONCLUSIONS

In summary, the results presented here provide a mechanistic explanation as to why there is soil C depletion after LUC increasing land use intensity in tropical soils: the huge reduction in the abundance of soil engineering invertebrates after LUC impair the ability of soil to physically protect SOM from decomposition inside stable aggregates, and therefore it is a primary mechanism controlling the soil C stocks' depletion across LUC in highly weathered soils. An average reduction of 89% in the density of the soil macrofaunal community was observed when sugarcane replaces pasture, and a loss of 39% in the diversity of macrofauna groups. Our findings showed that, over a range of soil textures (16 – 66% clay), such biodiversity loss was strongly correlated with the destabilization of soil structure across LUC with a gradient of land use intensity, whereas soil texture was not so. These observations consistently indicate that the abundance of detritivore soil animals, especially earthworms and termites, may be a significant predictor of soil structure transformations across LUC in tropical environments. Moreover, the sharp reduction in the abundance of earthworms was strongly positively correlated with the decrease in intra macroaggregate-associated C after LUC. As a result, after more than 20 years of sugarcane crop there were losses of 40 and 35% of C and N stocks, respectively, resulting in a rate of C emission of $1.3 \text{ Mg ha}^{-1} \text{ yr}^{-1}$, caused by the respiration of SOM from C4-cycle plants. This C loss primarily occurred in the macroaggregate-associated C, as a result of the faster macroaggregate turnover under sugarcane.

In addition, although more validation is needed, our results suggest that the humification index based on LIF spectroscopy of macroaggregate-occluded microaggregate samples may be a direct measure of the macroaggregate turnover and strongly simplify the measurement of macroaggregates turnover.

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