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CENTRO DE ENERGIA NUCLEAR NA AGRICULTURA**

CAIO FERNANDES ZANI

**Evaluation of soil carbon stocks in response to management
changes in sugarcane production**

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**Evaluation of soil carbon stocks in response to management
changes in sugarcane production**

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I dedicate

*To my lovely parents,
Francisca and Manoel, and
my brother Rafael, who
always was my example of
patience and commitment!*

*To my little girl Arlete, for
always being by my side
boosting me in every time!*

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“Stay hungry. Stay foolish”

Steve Jobs

ABSTRACT

ZANI, C. F. **Evaluation of soil carbon stocks in response to management changes and irrigation practices in sugarcane production.** 2015. 108 p. Dissertation (M.S.) – Centro de Energia Nuclear na Agricultura, Universidade de São Paulo, Piracicaba, 2015.

Brazilian commodities, such as ethanol, are looking for sustainable production to suit the international market demands. An important parameter for assessing sustainability is the carbon (C) footprint calculation of the product. Thus, studies of the variations in soil C stocks on the ethanol production are essential. Studies in relation to land use change are already been developed; however information about parameters of management changes on the sugarcane production is needed. The aim of this research was to evaluate the soil C stock in response to two main management changes in sugarcane production: i) no vinasse to vinasse application (NV-V), ii) burned to unburned harvesting system (B-UB). We also evaluated soil C stock changes in a chronosequence irrigation practices (native vegetation (NV), sugarcane irrigated 4 years (I4) and 6 years (I6), a new management in semi-arid and drought regions in Brazil which also aims high yields. Modelling approaches in order to assess long-term effects were also analysed. The NV-V transition showed higher soil C stock for V system for topsoil layers 0-40 cm depth mainly due to the addition of organic compounds to the soil. Vinasse can also enhance biomass production and crop yield. The B-UB transition showed higher soil C stock in the UB system from 20 to 60 cm depth due to higher organic matter accumulation from the maintenance of the straw to the field. The cumulative soil C stock for 1 metre depth had an increase of 1.1 and 0.75 Mg C ha⁻¹ y⁻¹ in the NV-V and B-UB transitions, respectively. From modelling was observed that V and UB sites had an increase of soil C stock by 2150, being a difference of 2.8 and 23 Mg ha⁻¹ in the equilibrium state between NV-V and B-UB systems, respectively. In the irrigation practices, the I4 showed higher soil C stock than NV in the 20 to 40 cm; while I6 was lower than NV in the 50 to 100 cm depth. Simulated long-term analyses showed increase of topsoil C stock of 12 and 13 Mg ha⁻¹ for I6 and I4 area, respectively, compared to NV on 2100. The results in this study are pioneers in relation to soil C stock studies in the management transitions and irrigation practices. This information may be used as a basis for public policies decision which dealing of the land use and global warming.

Key-words: Carbon turnover. Brazilian commodity. Management Changes. Irrigation practices. Mathematical modelling.

RESUMO

ZANI, C. F. **Avaliação do estoque de carbono do solo devido à mudança de manejo no sistema de produção da cana de açúcar.** 2015. 108 p. Dissertação (Mestrado) – Centro de Energia Nuclear na Agricultura, Universidade de São Paulo, Piracicaba, 2015.

Commodities brasileiras, como o etanol, estão à procura de uma produção sustentável para atender às exigências do mercado internacional. Um parâmetro importante para avaliar a sustentabilidade é o cálculo da pegada de carbono (C) do produto. Assim, os estudos sobre as variações nos estoques de carbono do solo (ECS) sobre a produção de etanol são essenciais. Estudos em relação à mudança no uso da terra já estão sendo desenvolvidos; no entanto informações sobre parâmetros de mudanças de manejo na produção de cana de açúcar são necessárias. O objetivo desta pesquisa foi avaliar o ECS em resposta a duas principais mudanças de manejo: i) não vinhaça para aplicação de vinhaça (NV-V), ii) sistema queimado para não queimado (B-UB). Mudanças de ECS também foram avaliadas em sistemas de irrigação em cronoseqüência: vegetação nativa (NV), cana de açúcar irrigada por 4 anos (I4) e por 6 anos (I6). Modelagem matemática para avaliar o efeito a longo prazo também foi analisada. A transição NV-V apresentou maior ECS para o regime V em 40 cm de profundidade, devido principalmente à adição de compostos orgânicos ao solo. A vinhaça também pode aumentar a produção de biomassa e rendimento da cultura. A transição B-UB apresentou maior ECS no regime UB em 20-60 cm de profundidade devido ao acúmulo de matéria orgânica a partir da manutenção da palha no campo. O ECS acumulado para 1 metro de profundidade obteve um aumento de 1,1 e 0,75 Mg C ha⁻¹ y⁻¹ nas transições NV-V e B-UB, respectivamente. A partir de modelagem foi observado que os regimes V e UB obteve um aumento de ECS em 2150, sendo uma diferença de 2,8 e 23 Mg ha⁻¹ no estado de equilíbrio para os regimes NV-V e B-UB, respectivamente. Nas práticas de irrigação, o I4 foi superior ao NV nos 20 a 40 cm; enquanto que I6 foi inferior a NV na profundidade de 50 a 100 cm. As análises de simulação a longo prazo mostraram um aumento de ECS de 12 e 13 Mg ha⁻¹ para as áreas I6 e I4, respectivamente, em comparação com NV em 2100. Os resultados deste estudo são pioneiros em relação aos estudos de ECS nas mudanças de manejo e práticas de irrigação. Esta informação pode ser usada como base para a decisão de políticas públicas que lidam com o uso da terra e do aquecimento global.

Palavras-chave: Turnover do carbono. Commodity brasileira. Mudanças de manejo. Práticas de irrigação. Modelagem matemática.

Summary

1 GENERAL INTRODUCTION	17
REFERENCES	20
2 CESSATION OF BURNING AND VINASSE APPLICATION IN SUGARCANE MANAGEMENT HAS POSITIVE IMPACT ON SOIL CARBON STOCKS.....	22
Abstract.....	22
2.1 Introduction.....	23
2.2 Material and methods	26
2.2.1 Site selection and description	26
2.2.2 Management comparisons	30
2.2.2.1 No Vinasse (NV) vs Vinasse (V).....	30
2.2.2.2. Burned (B) vs Unburned (UB).....	31
2.2.3 Approach, sampling method and analyses.....	31
2.2.4 Soil analyses.....	32
2.2.5 Physical fractionation.....	34
2.2.6 Soil C stock.....	34
2.2.7 Century model description.....	34
2.2.8 Initialisation, parameterisation and assessment of the model	35
2.2.9 Long-term scenarios	37
2.3.0 Statistical analyses	37
2.3. Results.....	38
2.3.1 Soil C stock.....	38
2.3.1.1 No Vinasse (NV) vs Vinasse (V).....	38
2.3.1.2 Burned (B) vs unburned (UB)	39
2.3.2 Carbon in soil fractions	41
2.3.2.1 No Vinasse (NV) vs Vinasse (V).....	41
2.3.2.2 Burned (B) vs Unburned (UB).....	41
2.3.3 Modelling soil carbon	44
2.4. Discussion	47
2.4.1 Effects of vinasse application on soil C (NV vs V).....	48
2.4.2 Cessation of burning is better for soil C (B vs UB)	50
2.4.3 Fractions assessment.....	54
2.4.4 Modelling assessments	56
Acknowledgement	59
References	59
Supporting Information	68

3 IMPACTS ON SOIL CARBON STOCKS OF DIRECT LAND USE CHANGE FROM SEMI-ARID WOODLAND TO IRRIGATED BIOFUEL CROP IN BRAZIL.....	71
Abstract.....	71
3.1. Introduction	72
3.2. Material and methods	74
3.2.1 Description of the study areas	74
3.2.2 Approach, sampling method and analyses	79
3.2.3 Soil analyses.....	79
3.2.4 Physical fractionation	80
3.2.5 Soil C stock.....	81
3.2.6 Century model description	81
3.2.7 Initialisation, parameterisation and assessment of the model	82
3.2.8 Long-term scenarios	83
3.2.9 Statistical analyses	84
3.3. Results	84
3.3.1 Soil carbon stock.....	84
3.3.2 Carbon in soil fractions	87
3.3.3 Modelling soil carbon and long-term scenarios.....	89
3.4. Discussion.....	92
Acknowledgements.....	97
Supporting Information.....	105
4. FINAL CONSIDERATIONS	107

1 GENERAL INTRODUCTION

Bioenergy crops, such as sugarcane (*Sacharum officinarum L*), have noteworthy potential to cater for sustainability energy needs. In Brazil, sugarcane was brought around 1532 during the colonial period. Originating from New Guinea, it is considered a monoculture or perennial crop and has a highly effective mechanism to convert solar radiation into biomass. Conventional management requires “reforming” of the sugarcane field, where the stalks are removed normally by heavy mechanical practices, following the introduction of sprouts into the prepared soil. Throughout the interval between each tillage, called the ratoon stage, the cane stubbles are allowed to re-grow after each harvest without any tillage for a period (cycle) of about 6 years (depending factors such as location, weather, crop varieties, and practice used). Afterwards, the tillage is necessary since productivity of sugarcane tends to decline throughout the ratoon stages.

Brazil has optimum conditions to sugarcane grow and is currently among the major producers in the world. Nowadays, Brazil emerges as a lead producer whether through productivity or land area coverage (FAO, 2015). In this scenario, Sao Paulo state appears as a greater producer representing around 60% of the total production (UNICA, 2015). Nationally the total agricultural area for sugarcane grew by 50% during the period from 2005 to 2013 (FAO, 2015) and is still expected an increase expansion of sugarcane field of 6.4 Mha up to 2021 to attend future ethanol demand required (GOLDEMBERG et al., 2014). Brazil produces approximately 45% of the world’s sugarcane, putting it ahead of India and China (FAO, 2015). Moreover, the cultivation of sugarcane in Brazil is economically and socially important as well as has a key role in the politics of the country.

Traditionally sugarcane was used in sugar production, however recently it has been gaining ground in national and international markets for use in ethanol production, so that, at least half of the total sugarcane production in Brazil is used for this purpose (CONAB, 2015). This is happening mainly because of the global growing population which is projected to increase by another 40% by 2050 (UNITED NATIONS, 2014) and consequently due to seek for energy and food security. Due to this aspects, OECD/FAO (2013) reported that Brazil will remain leading the exporter of ethanol, with roughly 50% of world trade, as well as being considered the

second greatest producer of ethanol. According to the same source, by 2022, the use of sugarcane for ethanol production will be around 28% of the total world production.

The production of sugarcane ethanol has increasingly been considered to be the exemplar feedstock in terms economic and sustainability for the most of the energy crops in the world (GOLDEMBERG, 2007). The biofuels as sugarcane ethanol may provide several environmental benefits, being responsible also for growth in aspects social and economic (WALTER et al., 2011). Besides that, the widespread acceptance of ethanol is the associated lower greenhouse gas (GHG) emissions during the whole life cycle and its use compared to the gasoline. According to Cerri et al. (2010), between 2005 and 2009, the use of ethanol as fuel has resulted in total GHG savings of total 232 Mt of CO₂-equivalent. In this way, when compared to other non-cellulosic biofuel, it has been reported that biofuel from sugarcane has a better energy balance and greater potential to mitigate GHG emission (GOLDEMBERG, 2007; CRUTZEN et al., 2008; AL-RIFFAI; DIMARANAN; LABORDE, 2010).

In addition, diverse international protocols mention carbon (C) sequestration as an important parameter in sustainable systems and thus, studies on soil C stocks are required to understand C cycling, potential international trade of C credits and the life cycle of biofuel production. As ethanol from sugarcane is an important commodity in Brazil, it has been focus of studies investigating the parameters that determine the C footprint of ethanol. One of the key parameters determining the C footprint for ethanol is the changes in soil C stocks proposed by European Directive (EC, 2009).

In this context, some variables for calculating C footprint have been calculated and studied, for example soil C changes due to land use change (MELLO et al., 2014). However, information about the soil C stocks change in response to management changes used in sugarcane production are rarely researched, with many studies assuming the C footprint default values for this unknown, which may not reflect Brazilian reality.

Management practices to increase sugarcane yields while avoiding negative environmental impacts is a key aim for policy makers. In view of this, the current sugarcane management system has received considerable attention relative a two main practices: reduced biomass removal, including a transition from pre-harvest burning to an unburned system, and appropriate reuse or application of co-products from the ethanol cycle on the field e.g. vinasse. There is evidence that changing the

management to conservationist practices, can provide an organic matter accumulation to the soil and, in a long-term, an increase of soil C stocks and low GHG emissions (SIX et al., 2004; DAWSON; SMITH, 2007; MAIA et al., 2010).

New management system can also be an alternative to produce energy and food for growing population with benefits to the environment. Irrigation system can be used in areas due to the no-irrigation practices limit the crop survival, and have been pointed out with great potential increase yields (WACLAWOVSKY et al., 2010; MONTEIRO; SENTELHAS, 2014) and soil C stocks (FOLLLET, 2001; LAL et al., 1998).

In this way, the aim of this study was to assess the change in the soil C stocks in response to management changes in sugarcane production, and also in the promising irrigation management. In addition, including mathematical modeling to develop future scenarios to extrapolate the data, obtained in the field, to a global scale. The modeling techniques were also used to study the soil C turnover in the soils.

The cessation of burning and vinasse application (the two main management transitions currently used in sugarcane production in Brazil) were chosen to investigate changes in soil C stock at the same location/area (*in situ*), and also future scenarios using modelling approaches (*Chapter 2*). In addition, the irrigation system, a new management approach used in semi-arid and drought regions in Brazil, was studied in relation to changes of soil C stock and the promissory contribution in enhance ethanol production in these areas (*Chapter 3*).

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2 CESSATION OF BURNING AND VINASSE APPLICATION IN SUGARCANE MANAGEMENT HAS POSITIVE IMPACT ON SOIL CARBON STOCKS

Abstract

Bioenergy crops have increased worldwide in recent years mainly aiming to replace fossil fuels. They have been highlighted as a promising development towards addressing environmental concerns and mitigating emission of greenhouse gases (GHG). Sugarcane as a bioenergy crop has the potential to contribute to energy sustainability providing a renewable alternative which can substitute gasoline. Improved sugarcane management practices (e.g. reduced biomass removal, application of co-products) may be used to provide further mitigation through increase soil carbon (C) sequestration, and reduced GHG emission. The aim of this study was to evaluate the impacts of burning and vinasse application on soil C stocks in one representative area of sugarcane production in Brazil. We explored how transitions between conventional (burning) and improved (no burning, vinasse application) management practices might influence both soil C stock, and the C in soil fractions down to 1 metre depth. Additionally, the CENTURY model was used to evaluate the impact of vinasse application, burning, and a combination of these scenarios under sugarcane in the simulated long-term (>100 years). The cumulative soil C stock to 1 metre did not show statistical difference between conventional and improved sugarcane management system. However, the soil C in the vinasse system showed higher values at 0-40 cm depth compared to the no vinasse system. The overall soil C stock was higher at 0-20 cm in burned compared to unburned system, while unburned showed higher values 20-60 cm depth compared to burned. In the soil fractions, there was significant difference in C content of silt+clay fraction (SCF) <0.053 mm, being higher in unburned compared to burned system at 0-20 cm depth. Modelling indicated that cessation of burning had increased topsoil C by almost 40% in the simulated long-term. Furthermore, vinasse application showed positive effects on soil C stocks, although these were not important as burning in driving changes in soil C stock. These findings demonstrate the positive effects of improved sugarcane management practices on the C balance, and the important role they have to reduce the payback time for soil C stock debts sugarcane ethanol. In this regard, the results also must be taken into consideration when it comes to the C footprint of ethanol.

2.1 Introduction

The expansion of bioenergy crops has increased in recent years mainly aiming to replace fossil fuel use. Sugarcane as a bioenergy crop has the potential to contribute to energy sustainability providing a renewable alternative which can substitute gasoline. There is a global interest to expand its production, as well as other bioenergy crops, due to current environmental concerns and the importance to mitigate emissions of greenhouse gases (GHG).

Sugarcane is grown in more than 100 countries with Brazil being the lead producer both in productivity and in land area coverage. Brazil has approximately 10 Mha of land cultivated with this crop (FAO, 2015) with half being cultivated for ethanol production and half for sugar production (CONAB, 2015). São Paulo state is the largest producer of sugarcane, responsible for approximately 60% of the total production of Brazil (UNICA, 2015). This crop is important for Brazil's economy and is responsible for around 45% of the world's total bioenergy crop production (FAO, 2015). The use of biofuels such as ethanol is an effective approach to attain environmental benefits whilst also providing a social and economic growth for the country (WALTER et al., 2011).

It is predicted that land use change (LUC) to sugarcane in Brazil will be 6.4 Mha in order to achieve global biofuel production requirements by 2021 (GOLDEMBERG et al., 2014). There are, however, concerns that bioenergy-driven LUC may impact food security (through expansion into cropped land) (WHEELER et al., 2013). Furthermore LUC may also adversely impact on soil carbon (C) storage as land use change can erode soil organic C stocks. This can result in a payback time where the fossil fuel replacement benefits require time to offset any losses of the soil C stock namely the "carbon debt" (FARGIONE et al., 2008; MELLO et al., 2014). Although the future prediction and the most of LUC in Brazil have occurred under degraded pasture where the C payback time is low, being an average of 2-3 years (MELLO et al., 2014). The impact of LUC to sugarcane fields has the potential to have positive or negative impacts on soil C budgets depending on the previous and current management practices (WALTER et al., 2011). The effective management applied may help reduce losses of soil C stock or even increase rates of organic matter inputs and thus promote soil C accumulation (LAL, 2004; SMITH et al., 2012).

Management practices which can increase bioenergy crop yields whilst avoiding negative environmental impacts (increasing soil C accumulation and decreasing GHG emissions) are required (LAL, 2004). The current and expanding sugarcane areas have received considerable attention with regarding two key issues of management system: 1) reduced biomass removal, including a transition from pre-harvest burning to an unburned system, and 2) appropriate reuse or application of by-products from the ethanol cycle on the field e.g. vinasse. These strategies are the main management transitions within the sugarcane production system across Brazil.

The burning of sugarcane prior to harvest is a common practice in Brazil in order to facilitate manual harvesting and transport operations, however the Federal Government in Brazil have set a deadline of 2021 for the termination of this practice which can result in GHG and black C emissions, both processes associated with global warming and human health issues (CANÇADO et al., 2006). It was estimated that mechanised harvesting practices might account for 80% of all harvesting from 2014 in the main sugarcane producing regions of Brazil (CERRI et al., 2013). This increased use of machinery could lead to new issues such as soil compaction resulting in lower crop yields and consequently tillage more often (WALTER et al., 2014). Even so, cessation of biomass burning will likely to play an important environmental role in the future agricultural system for ethanol production (GALDOS et al., 2013).

Vinasse is largely produced from the sugarcane-ethanol process with each litre of ethanol produced generating around 13 litres (L) of vinasse. Considering Brazil has an ethanol production of about 27 billion L per year, a huge amount of this by-product has been produced and applied by irrigation (ferti-irrigation) on the sugarcane fields since it is the simplest and least expensive destination for the solution. Vinasse has a high organic content (chemical oxygen demand-COD, 50-150 g L⁻¹), potassium (K) content (~2000 mg L⁻¹) (CHRISTOFOLETTI et al., 2013) and moderate amounts of nitrogen (N), calcium, phosphorus (P) and magnesium (at 0.28, 0.33, 0.10 and 0.13 kg m⁻³, respectively) (de RESENDE et al., 2006). These additions may improve soil fertility and soil quality, thereby reducing requirements for chemical fertiliser (LAIME et al., 2011). According to Fronzalia (2007) and Junior et al. (2008) around 80% of São Paulo state uses vinasse ferti-irrigation in sugarcane production. Despite of the benefits provided by vinasse, its application has been identified as the cause of environmental issues e.g. salinisation

(CHRISTOFOLETTI et al., 2013) as well as GHG emissions due to its storage and transportation besides the application on the soil (OLIVEIRA et al., 2013; 2015). These issues associated to the great quantity of this co-product suggest that the effects of vinasse use must be considered more holistically.

Certain management strategies have been reported to potentially increase soil C stocks; the conversion from a burned to an unburned system has been shown to accumulate C at a rate of $1.5 \text{ t C ha}^{-1} \text{ y}^{-1}$ in topsoil (30 cm depth) (GALDOS et al., 2010b; CERRI et al., 2011) though these management assessments were in different areas. The vinasse application also has suggested an increase of $0.25 \text{ t C ha}^{-1} \text{ y}^{-1}$, also for topsoil layers (de RESENDE et al., 2006). Nevertheless, few studies have focused on soil C stocks for deep layers, which may affect the whole system. Impacts of the land use and management change could also have an important effect on soil C distribution within particle-size classes of the soil (CHRISTENSEN, 2001) while association of soil organic matter (SOM) with different soil particle sizes and mineralogical composition would differ also in structure and functions (CHRISTENSEN, 1992), following management change.

The majority of research on sugarcane plantations has to date focussed on LUC and detrimental impacts due to the GHG emissions (FARGIONE et al., 2008). While some studies have assessed management impacts on soil C budgets (BRANDANI et al., 2014), management changes have not been examined at the same location, and few have assessed quality of C accumulated in soil particle-size fractions. The aim of this study was to address the impacts of changes in the management of sugarcane production, specifically cessation of burning and vinasse application. We explored how transitions between these management practices might influence soil C stocks, and C content in particle-size soil fractions, down to 1 metre depth in one representative area of Brazil. Additionally, we use a process-based model (CENTURY) to evaluate if either, vinasse application, burning, cessation of burning or a combination of these scenarios under sugarcane could result in long-term changes in soil C stock. Process modelling is a valuable tool for insight into simulated long-term effects and prediction for future scenarios. It may improve understanding and aid decision-making as well as provide targets for sustainable management.

2.2 Material and methods

2.2.1 Site selection and description

A paired-site approach was used to examine changes in management practices, with the previous and the current management in the same area. A pairing was only deemed suitable if it had identical soil type, climatic conditions and initial sugarcane cultivation duration under previous management (used as reference area). All study areas are with sugarcane under conventional tillage which use conventional tillage (ploughing and disking in the soil concurrently with sub-soiling). There are usually 4-6 harvest procedures (ratoon stage) of sugarcane after the planting of the crop. Throughout this stage the cane stubbles are allowed to re-grow after each harvest without any tillage. However, the rep-planting following by tillage, is necessary because continuous ratoon stage tends to decline the sugarcane productivity.

Two locations were identified that fulfilled all the above criteria for no vinasse vs vinasse (NV-vs-V) and burned vs unburned (B-vs-UB) comparisons. All four sites were located in Ourinhos, São Paulo state ($22^{\circ} 59'S$, $49^{\circ} 52'W$, 492 m above sea level) (Figure 1).

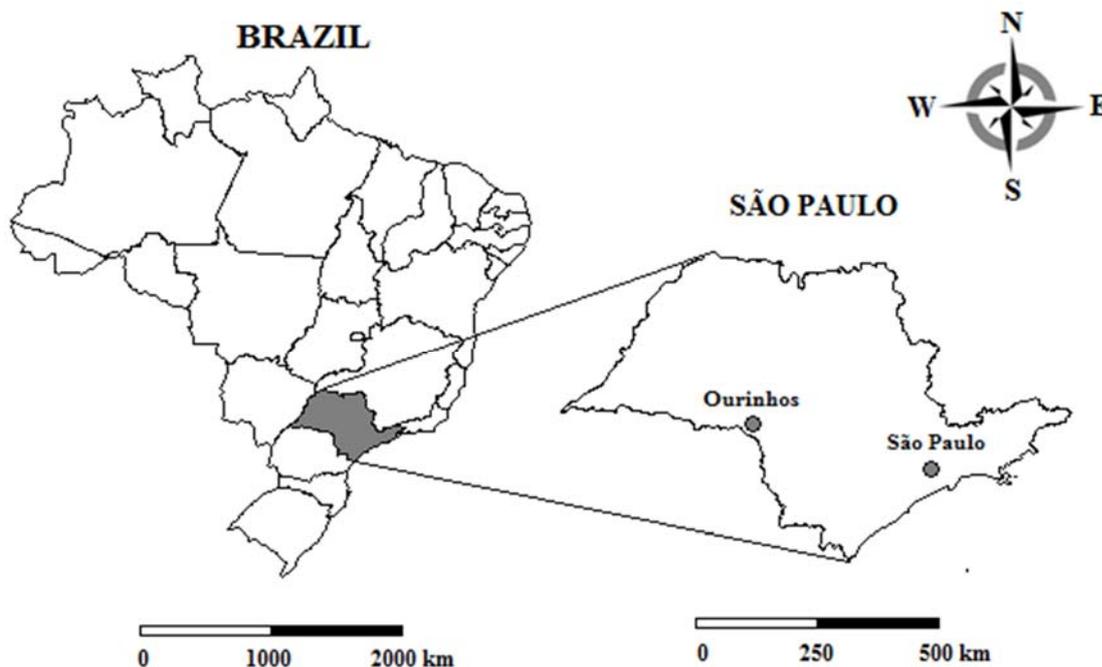


Figure 1 - Location of the study area in Brazil and São Paulo state. São Paulo city is the Capital of São Paulo state

The climate of the region is classified as tropical savanna with hot humid summers and cold dry winters according to the Köppen classification (Cwa tropical). The rainfall season is concentrated in the Spring-Summer (October to April) while the dry season, Autumn-Winter are comprised between May to September. The annual precipitation and temperature are 1321 mm and 22.8°C, respectively (Figure 2).

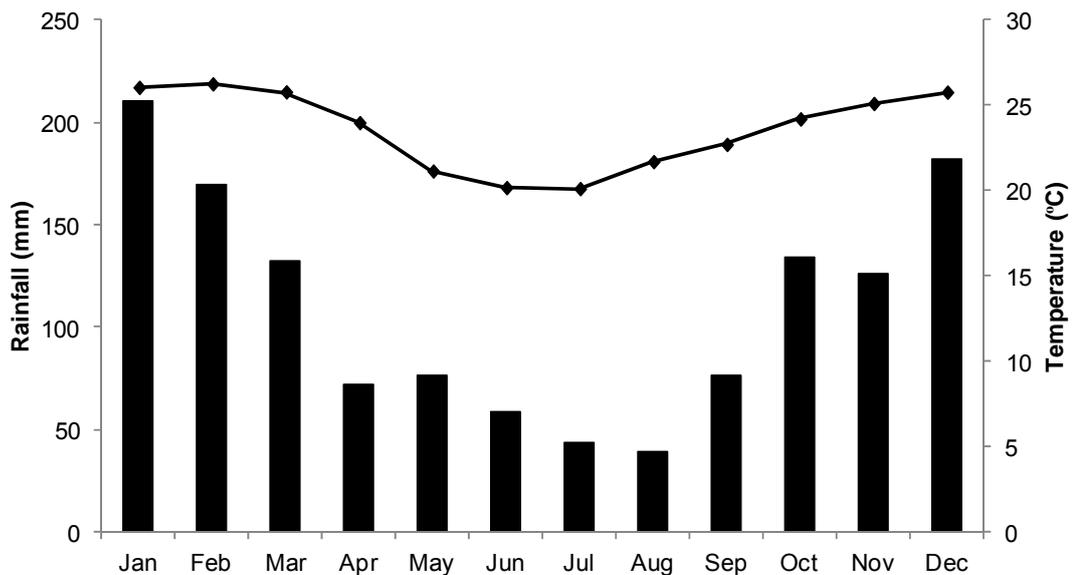


Figure 2 - Average monthly rainfall and temperature distribution in the region of Ourinhos-SP between 1961 to 2013

It was an average of 52 years of recorded weather (1961-2013) from the Instituto Nacional de Meteorologia (INMET) located in Presidente Prudente (170 km far from the areas) and from the São Luiz mill (Ourinhos). Both weather stations had monthly data collected. The soil at sites was classified as Rhodic Ferralsol (FAO, 2014) and Rhodic Eutrudox (SOIL SURVEY STAFF, 2014) and soil properties are shown in the Tables 1 and 2.

Table 1 - Soil textural properties and bulk density in the no vinasse (NV) and vinasse (V) area, up to 1 metre depth

Reference area	Depth cm	Sand	Silt	Clay	Texture (SBCS)	Bulk density
						g cm ⁻³
						g kg ⁻¹
No vinasse application (NV)	0-10	99	205	696	clayey.	1.1 ± 0.01
	10-20	91	209	700	clayey	1.08 ± 0.01
	20-30	83	166	751	clayey	1.12 ± 0.00
	30-40	89	203	708	clayey	1.14 ± 0.02
	40-50	100	278	621	clayey	1.04 ± 0.04
	50-60	92	204	704	clayey	1.04 ± 0.02
	60-70	119	195	686	clayey	0.99 ± 0.02
	70-80	116	205	679	clayey	0.95 ± 0.02
	80-90	112	186	702	clayey	0.95 ± 0.02
	90-100	92	195	713	clayey	0.93 ± 0.03
Vinasse application (V)	0-10	297	88	615	clayey	1.10 ± 0.00
	10-20	278	96	626	clayey	1.18 ± 0.00
	20-30	294	86	619	clayey	1.22 ± 0.00
	30-40	191	117	692	clayey	1.15 ± 0.03
	40-50	177	126	697	clayey	1.00 ± 0.01
	50-60	167	95	738	clayey	0.90 ± 0.03
	60-70	176	163	661	clayey	0.95 ± 0.02
	70-80	163	128	709	clayey	0.97 ± 0.02
	80-90	170	134	696	clayey	0.93 ± 0.03
	90-100	165	90	745	clayey	0.95 ± 0.01

SBCS: Brazilian soil classification system.

Table 2 - Soil textural properties and bulk density in the burned (B) and unburned (UB) area, up to 1 metre depth

Reference area	Depth	Sand	Silt	Clay	Texture (SBCS)	Bulk density
	cm	g kg ⁻¹				g cm ⁻³
Burned (B)	0-10	123	131	746	clayey	1.00 ± 0.00
	10-20	119	123	758	clayey	1.02 ± 0.00
	20-30	104	151	745	clayey	0.94 ± 0.00
	30-40	115	146	739	clayey	0.99 ± 0.04
	40-50	130	112	758	clayey	0.92 ± 0.02
	50-60	123	126	751	clayey	0.91 ± 0.03
	60-70	134	154	712	clayey	0.87 ± 0.02
	70-80	167	96	737	clayey	0.86 ± 0.03
	80-90	145	146	709	clayey	0.85 ± 0.02
Unburned (UB)	90-100	131	125	743	clayey	0.82 ± 0.03
	0-10	143	115	741	clayey	0.95 ± 0.00
	10-20	133	121	746	clayey	0.92 ± 0.00
	20-30	124	130	746	clayey	0.94 ± 0.00
	30-40	163	175	662	clayey	0.92 ± 0.02
	40-50	163	126	711	clayey	0.93 ± 0.03
	50-60	159	102	738	clayey	0.90 ± 0.03
	60-70	167	124	709	clayey	0.88 ± 0.03
	70-80	175	124	701	clayey	0.91 ± 0.02
	80-90	187	131	682	clayey	0.90 ± 0.03
90-100	166	103	731	clayey	0.89 ± 0.02	

SBCS: Brazilian soil classification system.

Mean clay content was 69% and 70% for NV-vs-V and B-vs-UB sites, respectively. The typical native vegetation in the region is seasonal semi-deciduous forest with Atlantic forest biome, comprising a transitional region between the Atlantic rainforest and Cerrado vegetation as supported by Franco et al. (2015) who sampled soil under native vegetation with the same soil type of the sugarcane areas, Rhodic Eutrudox.

2.2.2 Management comparisons

2.2.2.1 No Vinasse (NV) vs Vinasse (V)

The NV-vs-V paired sites were located in the oldest cultivated area (38.63 ha) of the mill. This area has been cultivated with sugarcane since 1958 when LUC from pasture occurred making the plantation 55 years old at establishment. Previously as soon as native vegetation was cleared, the area was pasture for at least 10 years. Since 2004, 12.72 ha has received vinasse applications (V site), whereas the remaining area 25.91 ha has continued to receive a traditional mineral fertiliser application (NV site) (Figure 3).

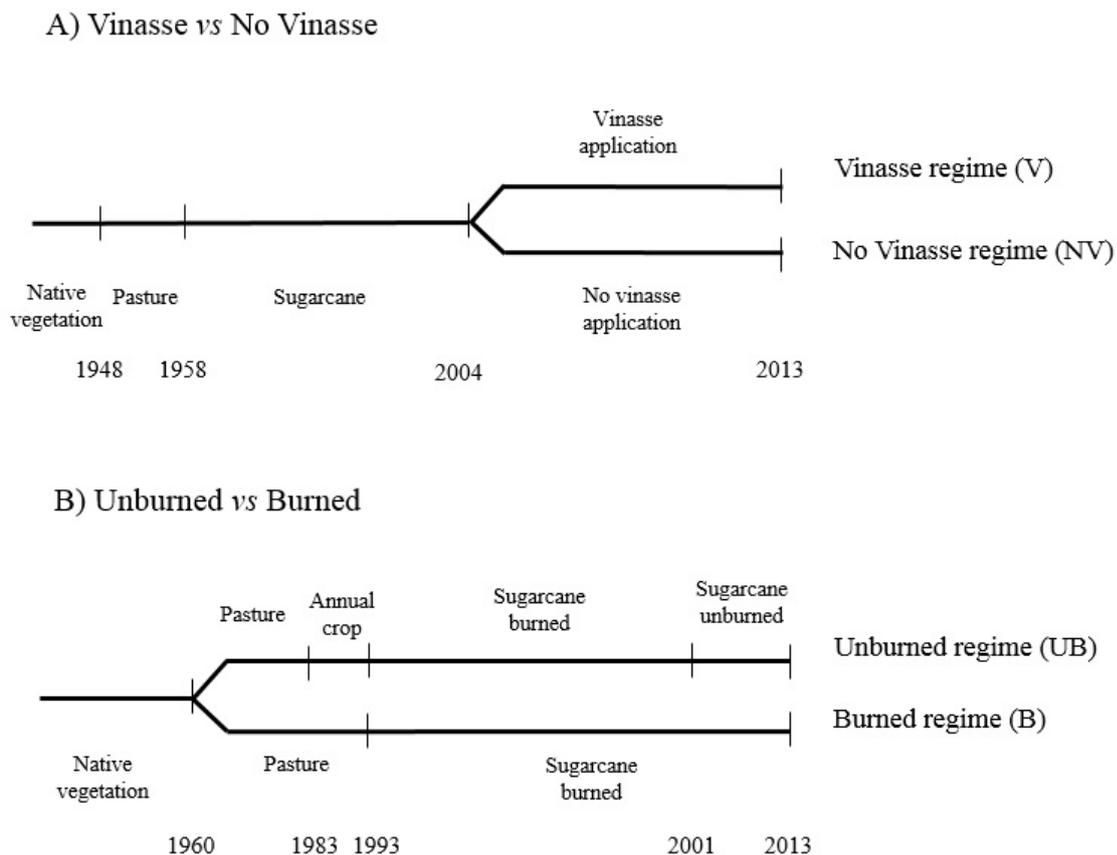


Figure 3 - Timeline of land use and management changes at each site A) No Vinasse vs Vinasse, B) Burned vs Unburned

The initial management system of sugarcane was burning followed by manual harvesting at both sites. In 2008 and 2009 mechanised harvesting was introduced to the V and NV sites, respectively, and last tillage occurred at both in 2011 i.e. second ratoon. N application was made in 2013 at a rate of 164 kg ha⁻¹ and 192 kg ha⁻¹ for V and NV, respectively. P was applied in January 2012 at a rate of 120 kg ha⁻¹, at both the V and NV site; this was in first stage of the crop cycle after tillage (plant stage).

The major difference in the fertiliser application between the study sites is in relation to K application. In the NV site, KCl is applied as an inorganic fertiliser at a rate of 520 kg ha⁻¹ at the time of sugarcane planting, whilst the vinasse was applied in the V site each year since 2004 at a mean of 268 m³ ha⁻¹. The amount of vinasse applied in the field is variable, and it is depending mainly on the chemical analyses of the vinasse and the soil exchangeable of K, as suggested in CETESB (2006). Mean K content usually found in the vinasse varies from 0.84 to 1.8 g L⁻¹ which corresponds to between 220 and 480 kg of K ha⁻¹ (OLIVEIRA et al., 2013; 2015).

2.2.2.2. *Burned (B) vs Unburned (UB)*

The B-vs-UB paired sites were located in an area of 30.5 ha which was previously forest and then converted to pasture in 1960. Both sites were converted to sugarcane in 1993, however, UB site had 10 years of annual cropping prior to sugarcane. Until 2001 the whole area was harvested using burning methods. In 2001 the plantation was split such that one area remained under burning management system (B site, 15.48 ha) whilst the other area had mechanised harvesting system introduced (UB site, 15.02 ha).

The B and UB sites were cultivated by conventional tillage with mineral fertilisers, with the last tillage being in 2008 and 2011, respectively. For both sites the most recent fertiliser application at a rate of 86 kg N ha⁻¹ in 2009. However, during planting the B site also received 119 kg P ha⁻¹ and 521 kg K ha⁻¹, whereas the UB site received 494 kg K ha⁻¹ with no P addition. The histories of each site, including the management practices and the previous land use, are showed in Figure 3.

2.2.3 *Approach, sampling method and analyses*

The experimental and sampling approach followed the schematic suggested by Cerri et al. (2013) and utilized by Mello et al. (2014) (Figure S1) with the exception

that, more soil layers were sampled in this study. The sampling site covered approximately 1 ha (100 × 100 m) and consisted of nine trenches distributed in a 3 × 3 grid with their centres spaced at 50 m apart. Three of the trenches were sampled in 10 cm increments to 1 m (deeper trenches, 120 × 120 × 120 cm). In each of the three deeper trenches soil samples were taken from two different soil walls to encompass between and within plant rows. The other six trenches were sampled to 30 cm depth (mini trenches 40 × 40 × 40 cm) with sampling being from one wall only in 10 cm increments. In each layer of each trench (deep and mini) two types of samples were taken: undisturbed samples were taken for bulk density assessments using stainless steel rings with a diameter and height of 5 cm (98.17 cm³ internal ring volume) (BLAKE; HARTGE, 1986) and also for determining the C and N content (78 samples), and disturbed samples were taken for chemical and physical analyses (60 samples). All soil samples were taken in July 2013 with a total of 138 samples for each of four study sites.

2.2.4 Soil analyses

The fresh mass of each sample was recorded in the laboratory. All samples were air-dried and sieved with a 2 mm mesh in order to remove stones and coarse roots fragments prior to soil analyses. Disturbed samples taken from deeper trenches were used to determine contents of sand, clay and silt as well as pH (CaCl₂) and the concentration of macro and micronutrients, according to Embrapa (1997) and Anderson and Ingram (1989). All of the layers (from 0 to 100 cm) were used for physical analyses (three replicates). While only the following specific depths (0-10, 10-20, 20-30, 40-50 and 90-100 cm) were used for chemical analyses (one sample consisting of three deeper trenches).

Soil moisture of all samples was determined by drying soil at 105° C for 12 hours followed by reweighing. To determine the total C and N only undisturbed samples were sieved through 100 mesh (0.150 mm) and ground to a fine powder prior to analysis. The total soil C and N were determined by dry combustion (NELSON; SOMMERS, 1996) using a Leco Truemat CN elementary analyzer (furnace at 1350° in pure oxygen). The analysis of physical and chemical soil attributes is presenting in Tables 1, 2, and 3.

Table 3 - Soil chemical analyses under different sugarcane management systems

Reference area	Depth	pH	P	K	Ca	Mg	H+Al	SB	CEC	BS
	cm									
No Vinasse application (NV)	0-10	6.1	13	2.0	64	22	16	88	104	84
	10-20	5.7	6	0.5	37	14	28	52	80	65
	20-30	5.3	4	0.1	20	8	22	28	50	55
	40-50	5.4	3	0.1	23	5	28	28	55	50
	90-100	5.6	1	0.1	17	2	20	19	39	48
Vinasse application (V)	0-10	4.0	57	7.9	28	11	79	46	126	37
	10-20	4.6	58	6.6	33	13	47	53	99	53
	20-30	4.5	77	3.5	14	6	58	23	81	29
	40-50	4.7	3	1.7	9	6	47	17	63	26
	90-100	4.4	3	0.3	9	5	42	14	56	25
Burned (B)	0-10	5.7	7	1.5	57	17	31	75	106	71
	10-20	5.3	5	0.6	37	15	28	52	80	65
	20-30	5.4	3	0.3	22	9	34	31	66	48
	40-50	5.8	2	0.1	18	6	20	24	44	54
	90-100	5.9	1	0.1	11	10	22	20	43	48
Unburned (UB)	0-10	4.4	7	1.5	21	11	52	33	86	39
	10-20	4.8	5	3.7	21	12	52	36	88	41
	20-30	4.8	4	0.5	12	7	34	20	54	37
	40-50	5.8	2	0.1	6	4	31	10	41	24
	90-100	4.7	2	0.1	2	2	25	4	29	15

SB=sum of bases, CEC=cation exchange capacity, BS=base Saturation

2.2.5 Physical fractionation

Physical fractionation techniques can be used in order to separate the soil organic matter and access the differences in C content on particles-size soil. The method used for physical fractionation was done according to Christensen (1985; 1992), which separates the particles sizes by dispersion, wet sieving and sedimentation. Basically this method does not consider aggregates, and includes only size and density separation of the uncomplexed organic matter and primary organomineral complexes (CHRISTENSEN, 2001).

The physical fractionation was carried out for the following depths: 0-10 cm, and 10-20 cm in triplicate, and at 40-50 cm, and 90-100 cm for all studied areas. For this 20 g of dry soil was sonicated in 60 mL of deionised water for 15 minutes. Subsequently, the sample was passed through a sieve of 270 mesh (0.053 mm) to separate the particle size fractions of the soil (Figure S2). The free organic matter (OMF) and sandy fractions (SF) both >0.053 mm, remained in the sieve which were separated by the density separation method utilising only distilled water. Silt and clay fractions (SCF) <0.053 mm, passed through the sieve and were quantified together. All fractions were dried, weighed and the total C content determined using the same procedures described in the “Soil analyses” section.

2.2.6 Soil C stock

Soil C stock was calculated by multiplying the soil C concentration (%) and bulk density (g cm^{-3}) for each 10 cm layer. As soil C stock is directly related to bulk density which can be altered by different management systems, it was necessary to adjust the mass of the soil layers being compared to a reference mass according to Ellert and Bettany (1995). The use of C content and bulk density data were used to make the comparison of soil C stocks between the paired sites in order to derive the equivalent soil mass across each management comparison.

2.2.7 Century model description

The Century model version 4.0 (PARTON et al., 1987) was used to simulate the main long-term changes in soil C stock for the upper 20 cm of the soil profile. Though the original model has been developed for prairies in North America it has been used widely for annual and perennial crops, forest and pastures

(PARTON; RASMUSSEN, 1994; CERRI et al., 2004; 2007; CONG et al., 2014). The models has also shown to be reliable results for sugarcane simulations and has a range of options for simulating management system (KEATING et al., 1994; VALLIS et al., 1996; GALDOS et al., 2009b; 2010a; BRANDANI et al., 2014).

Parton et al. (1987) described a detailed description of the structure of the model. In summary, the Century model is separated in several sub-models. Litter is separated into two fractions (metabolic and structural), and there are three soil organic matter pools (active, passive and slow), with each one having a different decomposition rate. Above and belowground litter pools and surface microbial pool are directly linked to the decomposition of surface litter. Different plant production submodels (grassland, crop and forest systems) are directly associated with at same SOM and nutrient cycling submodel (METHERELL et al., 1993).

2.2.8 Initialisation, parameterisation and assessment of the model

The initialisation of the model requires site-specific parameters including soil texture, bulk density, pH and N inputs. Weather data requirements include mean precipitation and minimum and maximum monthly temperatures for a long historical period. In view of this, the simulations were run using monthly data collected for 52 years of recorded weather (1961-2013) from a compilation of two stations: INMET and São Luiz mill.

The 'site.100' file used specific parameters from Franco et al. (2015) which sampled native forest vegetation (FNV site) nearby (15 km from the study sites). Similar soil texture was noted at both the sugarcane sites and below native forest vegetation (66% mean clay content for 20 cm depth). In order to initialise the first pre-cultivation conditions, the model was run to equilibrium state (7000 years) under native forest vegetation until 1958 at the vinasse manipulation site and until 1960 at the site where burning management was studied. The main focus of this study was not to make changes in the model; however some adjustments were made in order to achieve more accurate results. Model parameterisation for native vegetation was made adjusting the photosynthetic efficiency parameter (PRDX), using an average between default values of Century for Cerrado and Atlantic Rainforest (5299 g dry matter m⁻²). Minimum and maximum C-N ratios were altered in the model for leaves and fine branches in native vegetation, in accordance Franco et al. (2015). The average between default values of Cerrado and Atlantic rainforest, specified by

Century 4.0 were used for all the remaining parameters. A fire event was scheduled once every 120 years during the equilibrium spin-up, to simulate forest disturbance based on the premise that there are tree mortality as tree-fall and gap formation occurrence during this period (CERRI et al., 2004). Subsequent checks were performed to ensure the simulated above and belowground biomass was in line with measured data from published studies (CUNHA et al., 2009; VIEIRA et al., 2011). At the end of the equilibrium run simulated SOM levels, deforestation process was set up following procedures events parameterised by Cerri et al. (2004) which involve slash and burn.

As the soil sampled under native vegetation (FRANCO et al., 2015) and our sampled sugarcane fields were similar, model simulations used the same soil properties for all management system sites (66, 19 and 14% for clay, sand and silt, respectively). This helped to assess the influence of management change on soil C stocks independently from confounding variables. The site history of the two sugarcane sites were slightly different (Figure 3) and model simulations followed these systems. Specifically, soybeans and pasture management prior to sugarcane cultivation were included at one site assuming a typical management related to such practices in the region whereas the all other sites was converted to sugarcane from pasture. Default values specified for Century model were used to run soybean and pasture land uses. The results of these practices were also validated in relation to biomass productivity, above and belowground, results from simulating pasture were validated against Lilienfein and Wilcke (2003) and soybean growth was checked against values in Bordin et al. (2008), Walter et al. (2009) and Finoto et al. (2012). These equated to a mean of around 1.5 cattle ha⁻¹ stocking rate and 7 Mg ha⁻¹ aboveground biomass production for pasture and soybeans, respectively.

Afterwards, the model was run with sugarcane parameterisation for each study scenario based on values developed and reported by Galdos et al. (2009b; 2010a), including fire events for manual harvest (burned sugarcane, remove 85% of dry matter, leaves and tops), green harvesting cultivation (unburned system) and fertiliser additions (vinasse) parameters. The sugarcane field tillage was carried out by “conventional” default tillage parameters specific at Century. Each simulation scenario was informed by land management history from the mill and local farmers, as summarised in Figure 3.

The accuracy of model simulations to estimate sugarcane growth was estimated by comparing simulated aboveground biomass with yields obtained in the field. It was assumed that the C content on sugarcane plant biomass is 44% (SPAIN; HODGEN, 1994; ROBERTSON, 2003) whereas water content in the mature stalk is 70% (BULL; GLASZIOU, 1975). Furthermore, C content in specific plant parts (leaves, stalks and roots) were checked against measured empirical data collected at similar sugarcane plantations by Silva-Olaya (2014). Finally, the correlation coefficient (r) and root mean square error (RMSE) were used to compare the simulated and measured soil C stocks, in accordance with Smith et al. (1997).

2.2.9 Long-term scenarios

After evaluation of the model outputs, simulations were extended to predict long-term effects up to 2150 where a near-equilibrium state was achieved for all management practices. Each sugarcane cycle was determined with 6 years growth following conventional tillage. The monthly weather conditions were based on the general average (1961-2013) regardless of potential climate change, variation in atmospheric CO₂ concentrations or other factors that may influence sugarcane growth and soil respiration.

Further simulations were performed to evaluate the influence of varying application rates of vinasse. This also included a combination of a burned system plus vinasse at high and low application rates. This scenario was simulated since there are still divergences in the laws relating to termination of burned systems in areas where the slope is higher than 12% and the mechanised harvesting is difficult to apply. We propose that under these scenarios, vinasse may be a useful management practice to minimise the effect of burning on soil C stocks.

2.3.0 Statistical analyses

Differences in cumulative soil C stocks between land management types at 30, 50 and 100 cm were tested using linear models (LM), as was the C content in soil fractions at 20 cm. Model residuals were inspected for normality.

Differences in soil C stock depth profiles between land management types were tested using a bootstrap re-sampling and loess regression with the R stats package (R CORE TEAM, 2014). For this, all soil C stock values are treated as independent

data points. Firstly, the combined land management data (e.g. NV-vs-V) was re-sampled by bootstrap with replacement ($n = 1000$). These data were then modelled using loess regression and the bootstrap samples used to generate 95% confidence intervals around a modelled soil C profile. This represented the null hypothesis that there was no difference between the land management types. The data from the new land management only was then modelled using loess regression; if the modelled line for this soil C profile sits outside the confidence interval based on the null hypothesis, it can be inferred that soil C stocks are significantly different.

2.3. Results

2.3.1 Soil C stock

2.3.1.1 No Vinasse (NV) vs Vinasse (V)

Total cumulative soil C stock for the NV system was: 54.8 (± 0.3), 75.2 (± 0.9) and 119.5 (± 1.0) Mg C ha⁻¹ for 30, 50 and 100 cm layers, respectively, while for the V system it was 60.3 (± 0.7), 86.3 (± 1.7) and 130.4 (± 1.8) Mg C ha⁻¹ for the same layers, respectively. Although the cumulative C stock was higher under the V system, there was no statistical significance difference compared to NV for cumulative soil C stock at 30, 50 and 100 cm depth ($P > 0.05$).

Considering soil C stock in separate layers and independent data points, the V system showed higher values down to 40 cm depth compared to NV (mean of 20 and 16 Mg C ha⁻¹ respectively), higher value was found at 31.02 and 22.69 Mg C ha⁻¹, at 0-10 cm, under V and NV respectively. The NV system showed higher values of soil C compared to V at 60-70 and 70-80 cm layers and loess regression indicated that the C stocks were significantly different at these depths as well as for those topsoil layers (0-10, 10-20 and 20-30 cm depths) with V higher than NV (Figure 4).

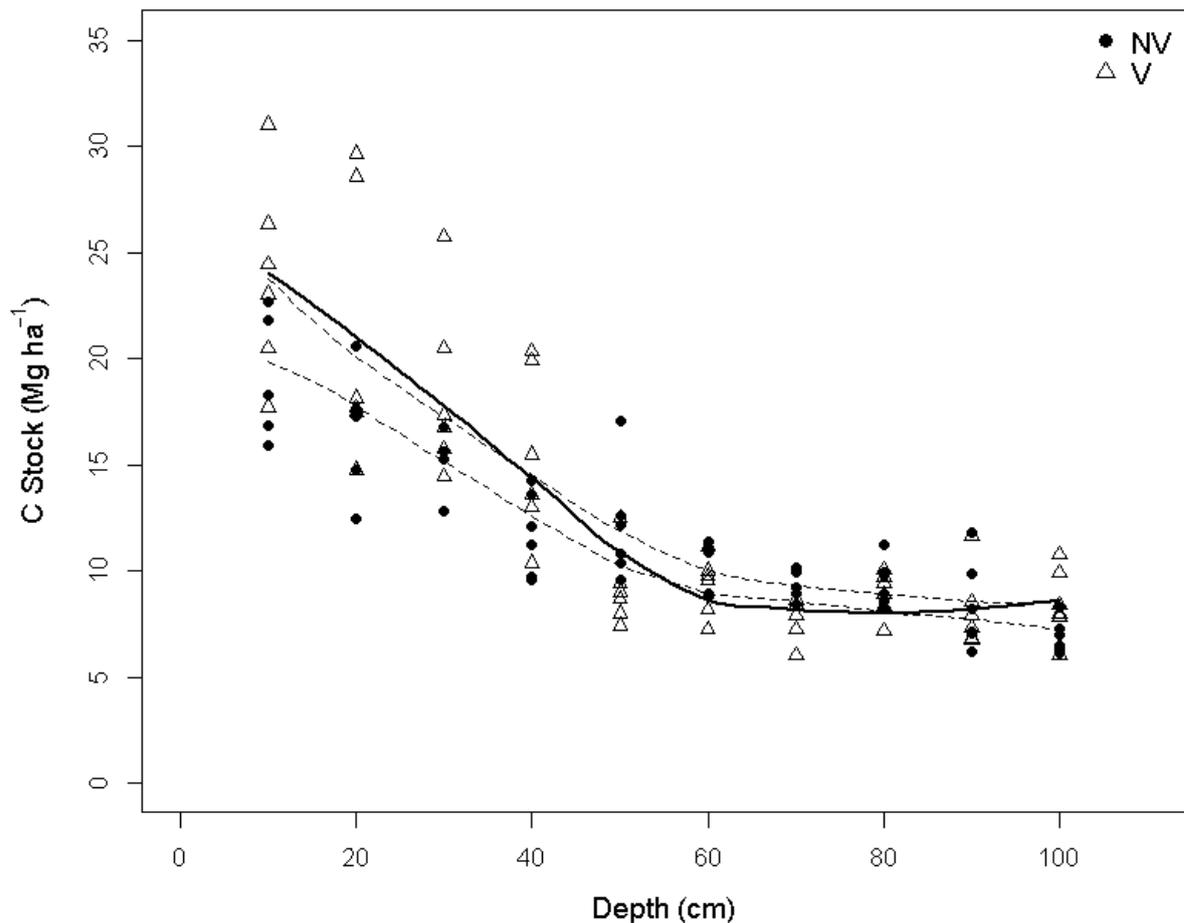


Figure 4 - Soil C stock (Mg ha^{-1}) between 0 - 100 cm depth in No Vinasse (filled circles) and Vinasse (empty triangles) land management systems/strategies. Dashed lines represent upper and lower bounds of 95% confidence intervals from bootstrapped ($n = 1000$) loess regressions of combined NV and V data; solid lines represents loess regression of soil C stocks in V only, if this line sits outside the confidence interval it can be inferred that NV and V are significantly different

Taking into consideration that vinasse was applied early in 2004, the cumulative C stock to 1 metre in V site had an increase of $1.1 \text{ Mg C ha}^{-1} \text{ y}^{-1}$ compared to NV system for 1 metre depth and of $0.5 \text{ Mg C ha}^{-1} \text{ y}^{-1}$ for 30 cm depth.

2.3.1.2 Burned (B) vs unburned (UB)

Total cumulative C stock for B system was: $63.1 (\pm 0.6)$, $81.8 (\pm 1.4)$ and $117.0 (\pm 1.5) \text{ Mg ha}^{-1}$ for the 30, 50 and 100 cm layers, respectively, while for UB system was $65.2 (\pm 0.4)$, $91.8 (\pm 0.9)$ and $121.7 (\pm 1.1) \text{ Mg ha}^{-1}$ for the same layers, respectively. Although the cumulative C stock was higher under the UB system at 0-30, 0-50 and 0-100 cm, there was no statistical significance difference compared to B for cumulative soil C stock ($P > 0.05$).

The overall mean of soil C stock was similar for the first 20 cm (mean of 23 and 22 Mg ha⁻¹ for B and UB, respectively), with higher value found under B system at 28.21 Mg ha⁻¹ and UB at 26.79 Mg ha⁻¹ at 0-10 cm depth. UB showed higher values from 30 to 60 cm depth compared to B, higher value found at 22.4 and 19.4 Mg ha⁻¹ at 20-30 cm, UB and B respectively. Loess regression indicated that the C stocks were significantly different at these depths (from 30 to 60 cm) (Figure 5).

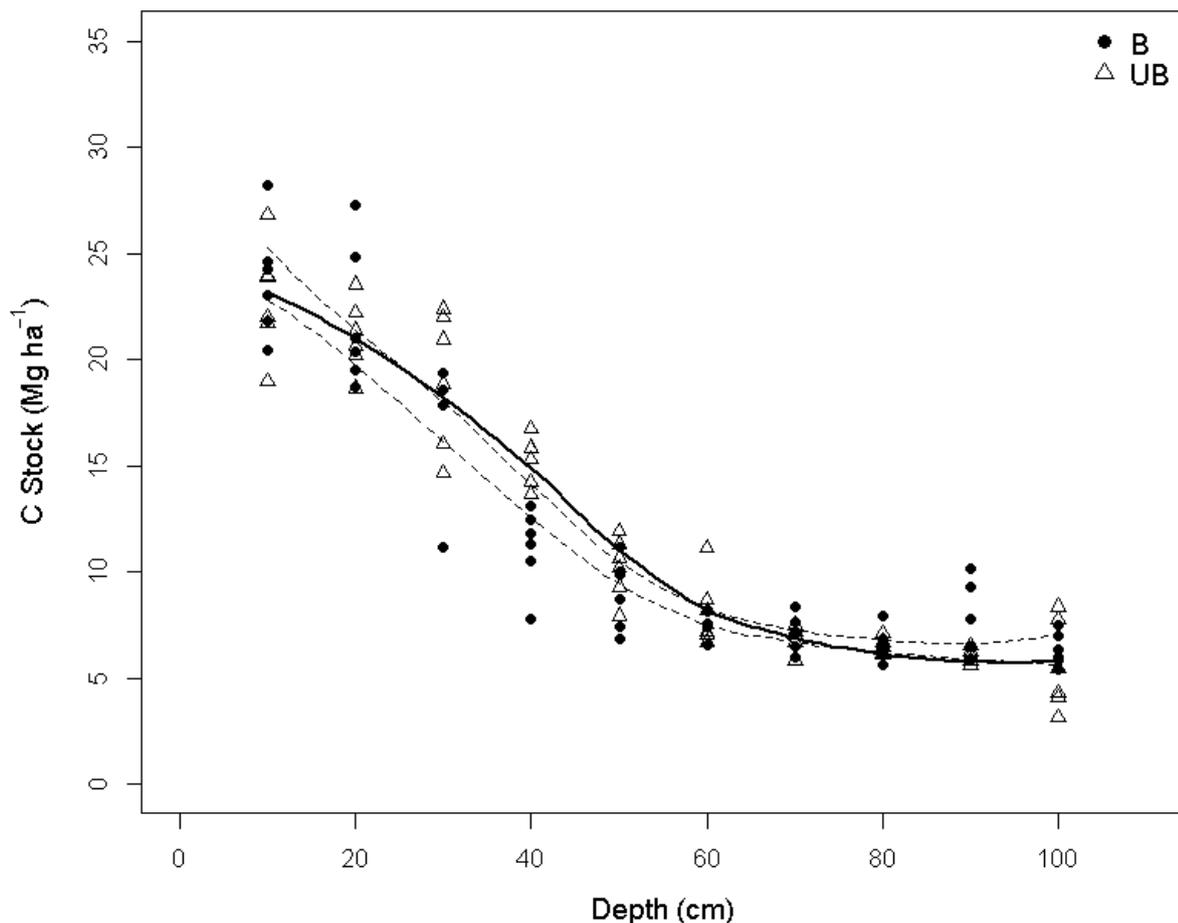


Figure 5 - Soil C stock (Mg ha⁻¹) between 0 - 100 cm depth in Burned (filled circles) and Unburned (empty triangles) land management systems/strategies. Dashed lines represent upper and lower bounds of 95% confidence intervals from bootstrapped (n = 1000) loess regressions of combined B and UB data; solid lines represents loess regression of soil C stocks in UB only, if this line sits outside the confidence interval it can be inferred that B and UN are significantly different

In these layers (from 30 to 50 cm), the C stock in UB system had an increase of 0.75 Mg C ha⁻¹ y⁻¹ compared to B system, whereas C stocks at depth intervals where significant differences were not evident (0-20 and 70-100 cm), the increase rate was considered negligible.

2.3.2 Carbon in soil fractions

2.3.2.1 No Vinasse (NV) vs Vinasse (V)

The physical fractionation proved to be an efficient method in order to quantify the SOM fractions without significant losses of samples (Table S1), with the mean soil sample recovery found in this study ranged between 97% and 98% for both systems assessed.

NV system had higher C content values in the OMF in the 0-10, 40-50 and 90-100 cm depths, while the V system showed higher C content values at the 10-20 cm depth. V site had higher C content in SCF for the first 20 cm depth compared to NV site. C in SF seems unalterable at both systems for all layers assessed (Figure 6).

Taking into consideration the values for the top layers 0-20 cm, there was no statistical difference between V and NV systems, in any of fractions ($P > 0.05$).

2.3.2.2 Burned (B) vs Unburned (UB)

The B site had a slight trend of higher C content values in OMF and SF for 0-10 and 10-20 cm; however C in SCF had higher values of C content found at UB system for all studied depths. The C content in the OMF, SF and SCF for the depths 40-50 and 90-100 cm was similar between B and UB (Figure 7).

Considering 0-10 and 10-20 cm depths together (top 0-20 cm), there was only a statistical difference in the C content in SCF, with UB higher than the B system ($P < 0.05$) (Figure 7).

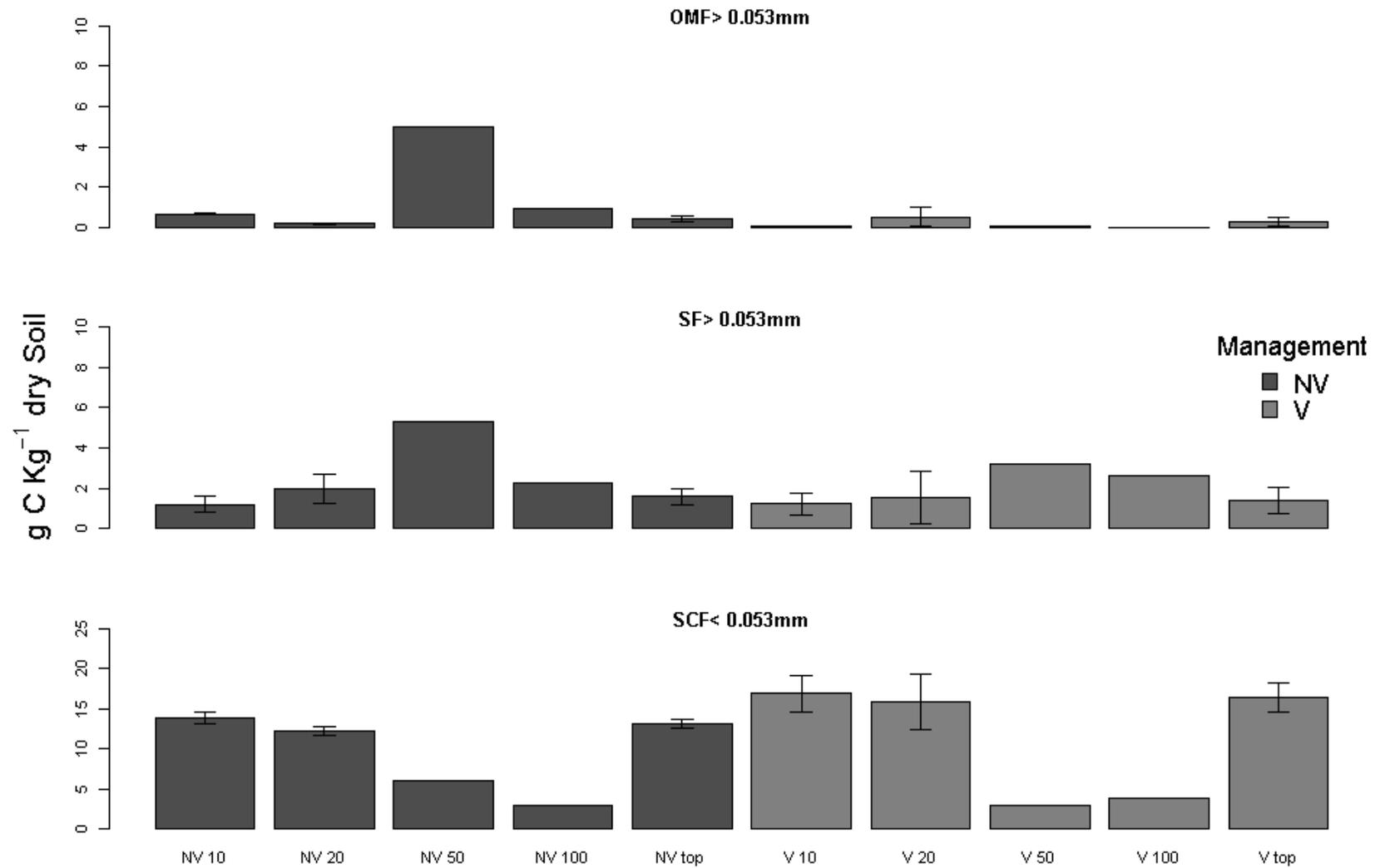


Figure 6 - Soil C content (g C kg⁻¹) in organic matter (OMF), sand (SF) and silt+clay (SCF) soil fractions for no vinasse (NV) and vinasse (V) application systems. Top means 0-20 cm depth. Vertical bars show ±1 standard error (n = 3)

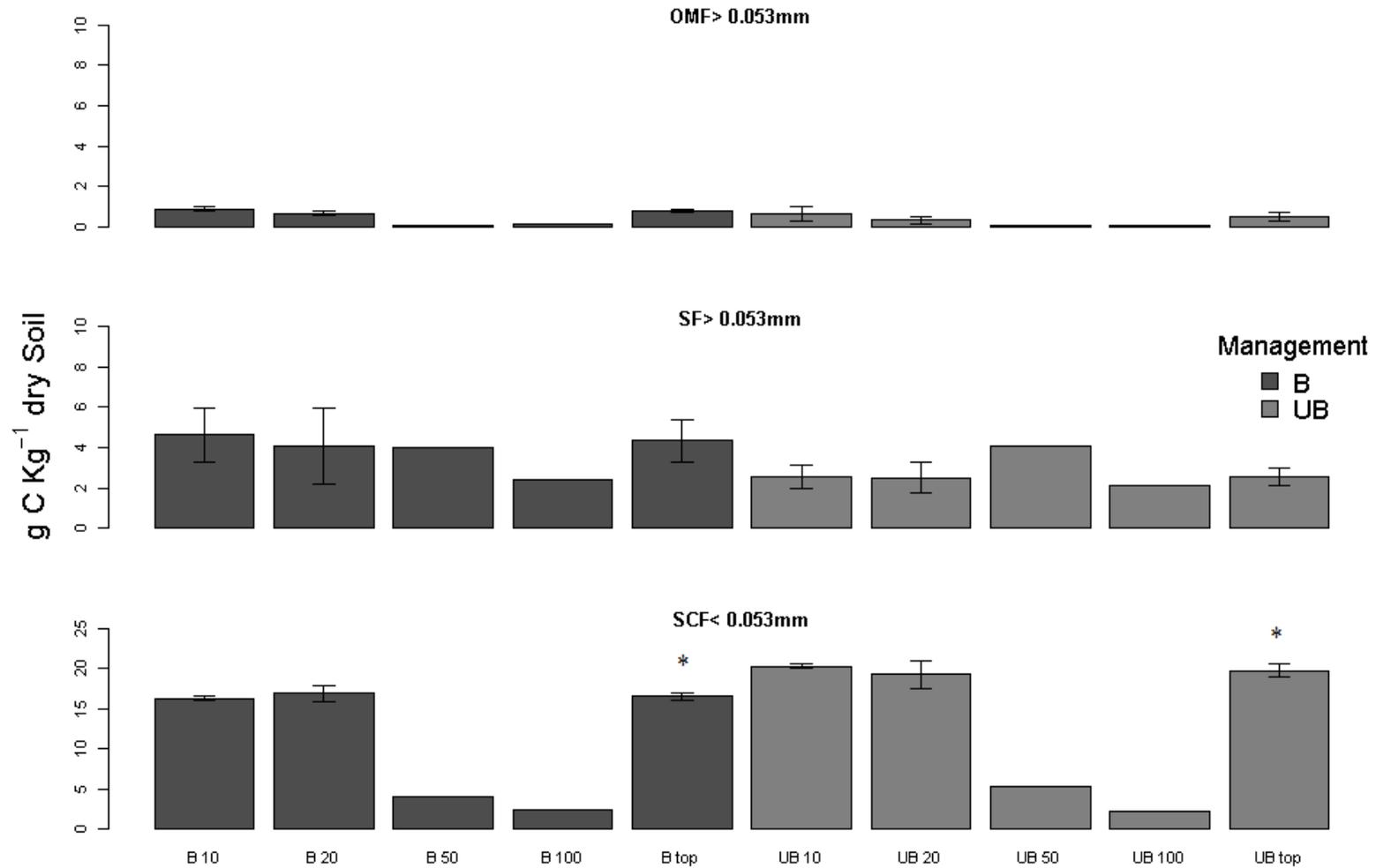


Figure 7 - Soil C content (g C kg⁻¹) in organic matter (OMF), sand (SF) and silt+clay (SCF) soil fractions for burned (B) and unburned (UB) systems. Top means 0-20 cm depth. Vertical bars show ± 1 standard error (n = 3). Asterisk denote significant difference (P < 0.05)

2.3.3 Modelling soil carbon

The model showed a good fit between simulated vs measured values at 0-20 cm depth for all sites assessed (r value = 0.90 and RSME = 1.72; Figure 8). The difference between simulated and measured values was less than 6% and no statistical difference was found between them, suggesting a good model performance. Both measured and modelled results showed that FNV had a higher soil C stock (53.60 and 52.55 Mg ha⁻¹ respectively) than sugarcane sites. The differences between values measured and simulated are 1.92, 0.89, 1.51, 2.65 Mg ha⁻¹ for the NV, V, B and UB sites, respectively (Figure 8).

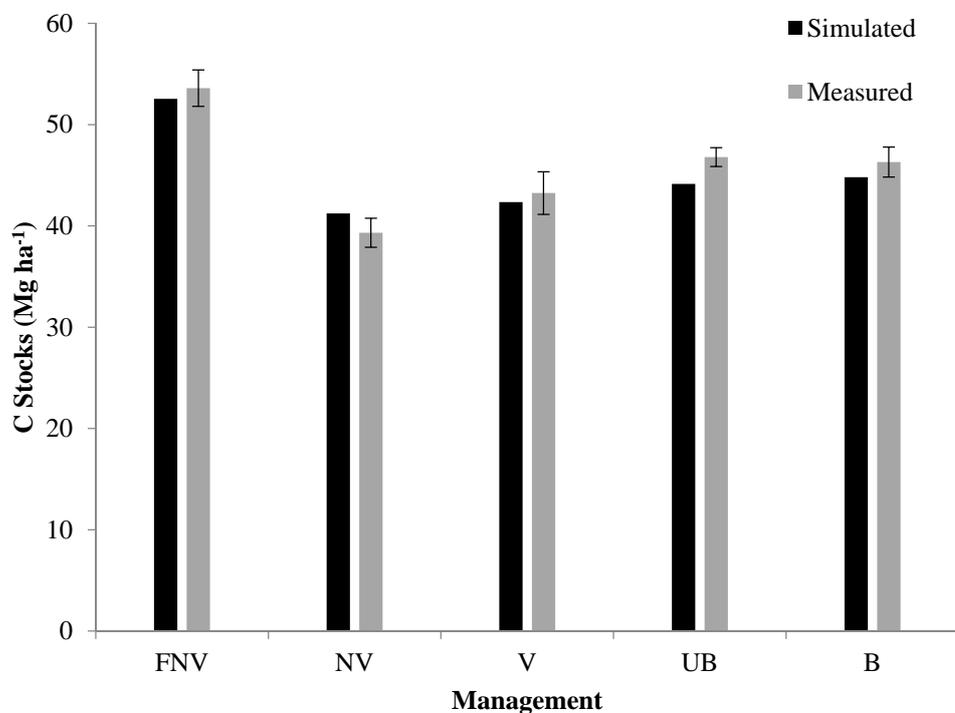


Figure 8 - Simulated and measured results for soil C stock (0-20 cm) under different sugarcane management systems (NV, V, UB and B) and native vegetation (FNV as measured by Franco et al. (2015)). Vertical bars show ± 1 standard error ($n = 12$)

Simulating LUC from native vegetation to pasture a mean soil C loss of 0.25 Mg ha⁻¹ y⁻¹ was observed (34 years under pasture). Greater losses were observed in the first 10 years post conversion (1 Mg ha⁻¹ y⁻¹). On the UB site, where pasture were replaced by croplands highest C losses were found, rate mean of 1.5 Mg ha⁻¹ y⁻¹. The mean soil C loss rate was variable for LUC to sugarcane from

pasture and depends of the sugarcane management system used. Soil C loss rate was observed from pasture to sugarcane when burning was used ($-0.25 \text{ Mg ha}^{-1} \text{ y}^{-1}$). The opposite trend was observed when sugarcane replaced annual crops with soil C gains of $0.35 \text{ Mg ha}^{-1} \text{ y}^{-1}$ even under burned system. Besides that, results from loess regressions were confirmed over the topsoil layers 0-20 cm; vinasse application has higher soil C stocks than NV site in the simulations as well (2.7%). In addition, the model also did not show a notable difference over the top layers for the B and UB comparison, showing values of 44.80 and 44.14 Mg ha^{-1} , respectively.

As the simulations were consistent with measured values (Figure 8), a simulated long-term modelling 137 year projection was made from 2013 to 2150 (Figure 9). In the V site the use of vinasse led to an increase of $0.15 \text{ Mg C ha}^{-1} \text{ y}^{-1}$ from 2013 up to 2150 in the soil C stocks, whereas NV increased by $0.13 \text{ Mg C ha}^{-1} \text{ y}^{-1}$. Thus led to a difference of 2.8 Mg ha^{-1} in the equilibrium state of both systems on 2150 (Figure 9a).

In contrast to measured results, greater differences were found between B and UB sites in the equilibrium state (2150). The increment in the UB site due to the maintenance of straw in top soil provided by machinery harvesting, led an increase of $\sim 0.12 \text{ Mg C ha}^{-1} \text{ y}^{-1}$ while the B site had a decrease of soil C stock of around $-0.05 \text{ Mg C ha}^{-1} \text{ y}^{-1}$ from 2013. This led to a difference of 23 Mg ha^{-1} in the equilibrium state between systems by 2150 (Figure 9b).

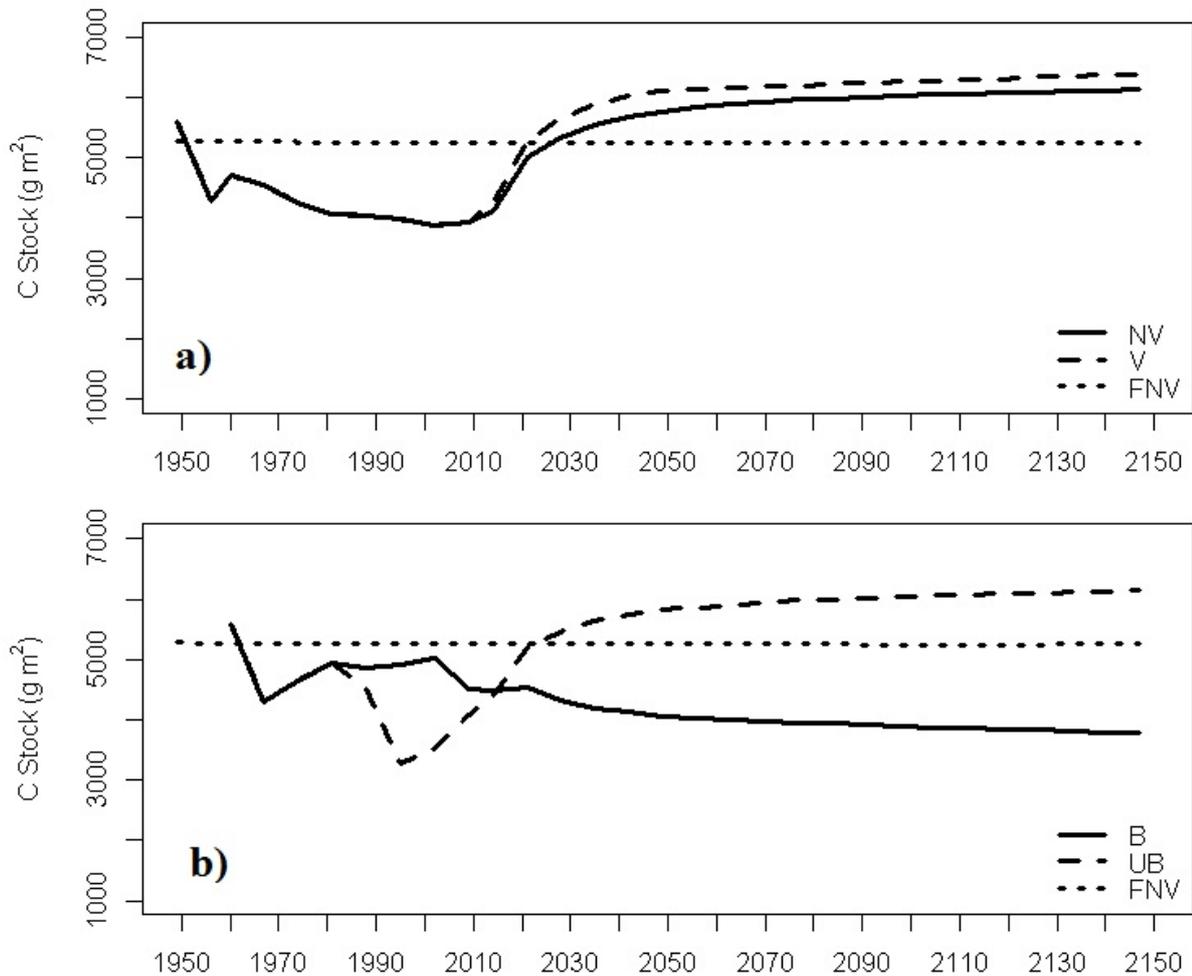


Figure 9 - Long term approaches over 137 years projection for a) NV vs V (no vinasse vs vinasse application) b) B vs UB burned vs unburned. FNV is the native vegetation from Franco et al. (2015)

From the simulations, we also found that the use of vinasse in a burned system might decrease the rate of soil C losses in relation to the system only burned (Figure 10a). There was an increase of 2.16 Mg C ha⁻¹ on the burned system under lower doses of vinasse in comparison to burned without vinasse. With high dosages, this increase of soil C may be double (average of 0.038 Mg C ha⁻¹ y⁻¹) resulting a difference of around 5 Mg C ha⁻¹. In the unburned case, the use of different dosages of vinasse had a similar pattern observed under burned. The increase rate from lower rates of vinasse was the same reported previously (0.02 Mg C ha⁻¹ y⁻¹) which result a difference of around 2 Mg C ha⁻¹. Nevertheless under unburned system, whether through low and standard doses had the similar soil C stock in 2150, while high dosages had an increase of 0.03 Mg C ha⁻¹ y⁻¹ (Figure 10b).

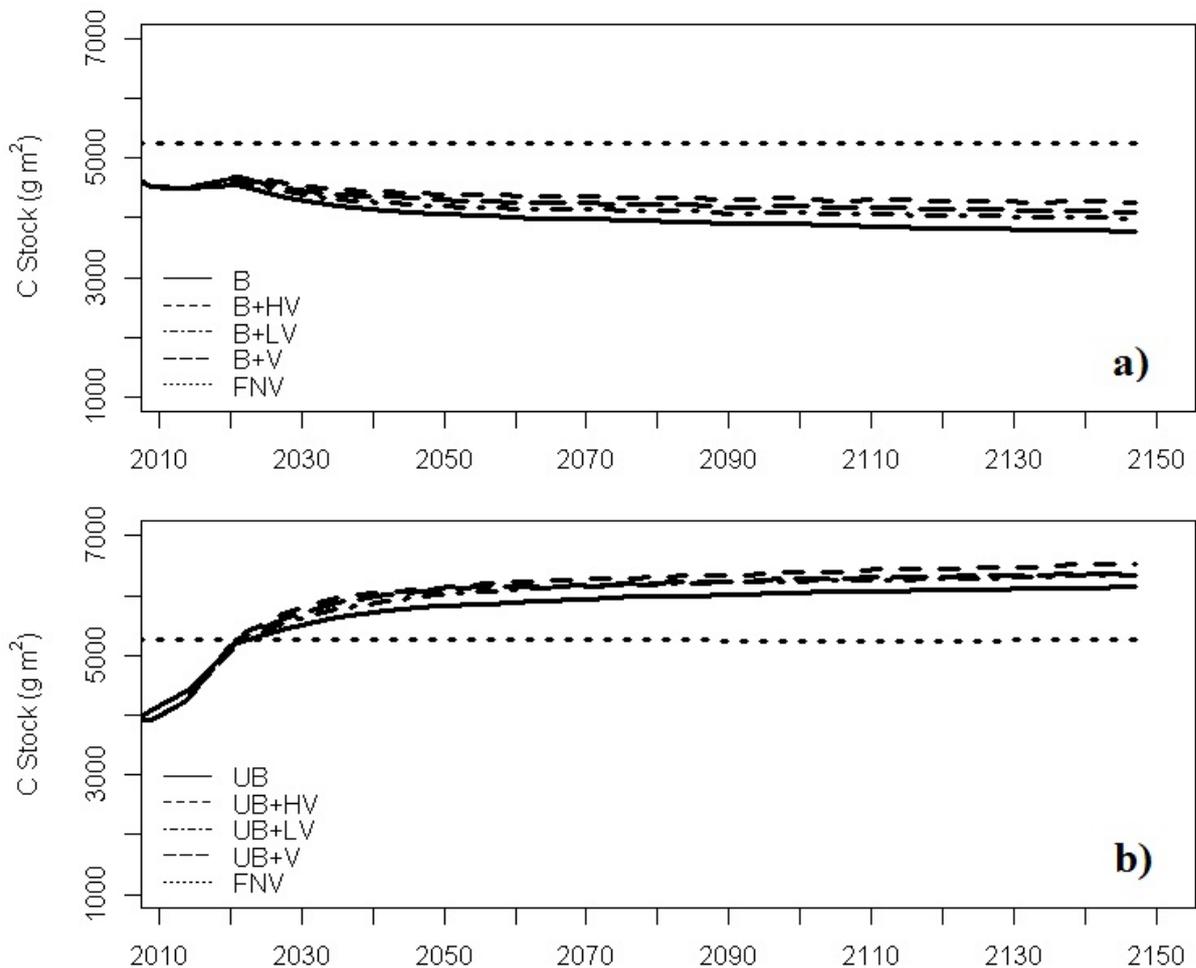


Figure 10 - Long-term approaches over 137 years projection for a) burned (B) and b) unburned (UB) plus different dosages of vinasse: high (HV), low (LV) and standard (V). FV is the native vegetation from Franco et al. (2015)

2.4. Discussion

Through conservation management techniques, Brazil has an opportunity to produce renewable fuels with limited C losses, particularly from soils. Additionally, appropriate management practices can limit the increase indirect LUC and consequently GHG emissions which coming mainly due to the LU and LUC (MELLO et al., 2014). Improved management might also achieve highest productivity which leads to increase soil C stock offsetting those emissions and also face the main issues as climate change and food security. It is important to highlight that potential changes on soil C stock can be influenced by other factors, such as climatic conditions and soil type.

2.4.1 Effects of vinasse application on soil C (NV vs V)

With sugarcane propagation for biofuel use expected to increase in the near future (GOLDEMBERG et al., 2014), by-products of ethanol production, such as vinasse, have received more attention. While vinasse has been considered as a serious environmental problem due to potential discharge into water bodies (CÓ JUNIOR; MARQUES; TASSO JÚNIOR, 2008). A recent evaluation by Prado et al. (2013) highlighted that vinasse application is not necessarily an environmental problem, and can in fact be used for several benefits. Since the 1980s applying vinasse as a fertiliser became a standard practice in order to lower costs associated with chemical fertiliser (LAIME et al., 2011). Beyond a better profitability due to reduced fertiliser costs, the use of vinasse is also related to improved soil fertility without environmental risk when applied at acceptable levels as suggested by CETESB (2006). Even so, the majority of research has focused on GHG emissions and relates to vinasse discharge as a pollutant (FUESS et al., 2014). Oliveira et al. (2013) reported an increase of CO₂ (almost tripled) and N₂O emissions from the soil due to vinasse application on sugarcane fields even though the emission factor found in this study was considered significantly less than IPCC prospects, and other studies has pointed that the amount of CO₂ emitted is a part of the closed sugarcane cropping cycle (CARMO et al., 2013) Few studies, however, have examined the potential of soil C stock accumulation due to vinasse application in the soil.

Our results show that the management change from NV to V application can provide greater soil C stocks for all layers down to 30 cm depth as observed using loess regression. This technique was used based on Kravchenko and Robertson (2011) who suggest that a more effective evaluation of soil C stock change is to assess each soil layer separately due to the potential variability on assessment. The evaluation of each soil layer with independent data points indeed showed that there is clearly an increase of soil C stock with vinasse application, especially for topsoil layers (0-10; 10-20 and 20-30 cm) where statistical differences were found (Figure 4). The increase noted only in topsoil layers was likely caused by increase organic matter inputs in the soil under vinasse application as found in a number of similar studies (de RESENDE et al., 2006; ARAUJO et al., 2009; ZOLIN et al., 2011; BRANDANI et al., 2014). Zolin et al. (2011) found that soon after the first year of vinasse application, there was an increase in C and K contents in the topsoil layers. Such elements also increased in our study and, although the organic matter from

vinasse may be readily decomposed by microorganisms, it is likely that the enhancement of soil fertility provided by vinasse application leads to higher soil C stocks.

The availability of nutrients, organic matter and water present in the vinasse applied to the soil surface might also have stimulated higher root biomass, only in topsoil layers (0-30 cm depth). In turn, an increased concentration of roots in the topsoil layers may have stimulated the increase in soil C stock through greater root turnover and exudates. Carmo et al. (2013) highlighted that despite the increase in GHG emission, a potential higher soil C stock may occur and a full cycle analyses is required. This gap was the stimulus to carry out the process modelling under conditions of burned system plus vinasse application, since our sampled vinasse areas were all under unburned system. In addition the most of studies showed higher emission under unburned system plus vinasse application (OLIVEIRA et al., 2013; CARMO et al., 2013) (these results are discussed in modelling assessments section; 2.4.4).

A further explanation for the increased C stock, may have been related to the enhanced biomass production and crop yield under vinasse application, which can have direct effects on soil quality and fertility through improved physical, chemical and biological properties (NEVES et al., 1983; JIANG et al., 2012; PRADO et al., 2013). Jiang et al. (2012) found that, after only three years under vinasse application, soil porosity was maintained or even increased, in addition to an increased aggregation of fine soil particles at 0-30 cm depth. The higher aboveground biomass production may also lead to an increased accumulation of straw on the field, which in turn could increase topsoil C stocks through enhanced decomposition, as reported by Galdos et al. (2009a). Increased yield was reported by de Resende et al. (2006) under sugarcane production with vinasse application, particularly in the first and second cycles, with an average increase of around 13%. This study suggested that the high yields are directly linked to the increase of exchangeable K, and perhaps reduced acidity and reduced Al^{3+} toxicity. In our field site, K content increased under vinasse application compared to the no vinasse system (Table 3).

In contrast to greater soil C under V for 0-30 cm depth, higher soil C stock was found in the NV at 50-60 cm and 60-70 cm depth. It suggested that vinasse is less likely to penetrate deeper soil layers, and therefore may not influence soil C stocks further down the soil profile. Secondly, the higher soil C stock found under no

vinasse system might be attributed to a likely high root growth at depth. Although the areas have the same history prior to vinasse application, it is also possible that differences in initial soil C stock in the subsoil may be related to spatial variability and position in the landscape. These aspects together might have influenced the lower soil C storage found in vinasse application site, at such depth interval.

In summary, the results suggest that vinasse application was beneficial for the soil and leads to a soil C accumulation during 9 years in the topsoil layers of $0.55 \text{ Mg C ha}^{-1} \text{ y}^{-1}$. This is more than double the rate found by de Resende et al. (2006) over 16 years ($0.25 \text{ Mg C ha}^{-1} \text{ y}^{-1}$). This significant increase in soil C stocks is an important part of C accounting in managed sugarcane plantation, and therefore is important to be considered in any future life-cycle analyses. On the other hand, despite the accumulation of $1.09 \text{ Mg C ha}^{-1} \text{ y}^{-1}$ found at 1 metre depth, there are uncertainties about the effect of vinasse below the topsoil layers (0-30 cm), and it is likely that this result is associated with root growth in the topsoil layer under vinasse application while in the no vinasse system, the root growth should occur deeper in the soil profile.

Further evaluation is required to determine optimum application rates of vinasse in order to enhance yields and fertility of the soil while ensuring minimal losses. Consequently through this enhance yield and fertility; the soil C stock will also increase. Where optimum rates exceed the supply in a given region, vinasse can also be used for alternatives end-uses (combustion, yeast production, livestock feed production, incineration) as reported by Christofolletti et al. (2013). Future studies should also aim to quantify how optimum application rates may influence soil quality and C budgets.

2.4.2 Cessation of burning is better for soil C (B vs UB)

As mentioned in the history of the management sites (Figure 3), the unburned site had only 12 years after its conversion, being the mainly issue for lower and not significant difference in the measured cumulative C stocks. IPCC suggests a timeframe of around 20 years to achieve a new equilibrium state for soil C stock after a LUC. Potentially the management change will follow the same trend. Besides that, the land use history of sites can be another important point that may have influenced the cumulative soil C stock.

The current UB site had many different crops previously (although mainly soybeans), for at least 10 years, while the B site remained unchanged from pasture prior to becoming a sugarcane field (Figure 3). This indicates that the initial soil C stock in the sites may not be the same before the introduction of sugarcane. According to Zinn et al. (2005) and Maia et al. (2010), croplands may entail in a higher losses of C due to intensity of land use (annual tillage). Whilst in the other hand, Maia et al. (2009) highlighted that well managed pastures may maintain or even have a great potential to increase soil C storage. In some cases the authors suggested that soil C stock might be similar to native vegetation areas. Hence, it is accepted that pasture has a greater capacity to maintain higher amount of C than croplands.

Conversely, Mello et al. (2014) pointed out that areas converted to sugarcane from pasture may have substantial losses of C but calculated the payback time did not require a substantial period (2-3 years) to replace the C lost during the LUC. On the other hand, the authors highlighted that areas converted from annual crops have an increase in soil C stock without any payback time. In our sites, even though both were in the same area, it is worthwhile to highlight that the B site had two LUCs, natural vegetation to pasture (1960) and pasture to sugarcane (1993). At the U site; the land use changed three times, natural vegetation to pasture (1960), pasture to croplands (1983) and croplands to sugarcane (1993). In view of this, after approximately 20 years with the use of sugarcane in both areas and taking into consideration the LUC factors found by Mello et al. (2014), the unburned and burned sites potentially had in the year of sugarcane introduction (1993) around 101.06 and 125.23 Mg ha⁻¹ of soil C stock in 1 metre depth, respectively. Values later confirmed and reported in the modelling approaches section.

Even with prior differences between B and UB, our recent approach using loess regression showed significant differences with higher soil C stock from 30 to 60 cm depth, after 12 years under UB system. It suggests that the conversion from burned to unburned sugarcane will lead to soil C accumulation also for deep layers. In contrast, our results did not find significant differences in the topsoil (0-20 cm) as evidenced in other studies (GALDOS et al., 2009a; CERRI et al., 2011; SIGNOR et al., 2014). It is presumed that disturbance during the preparation for replanting might play a key role by homogenising the topsoil layers (0-20 cm). The last soil disturbance in the UB site was only two years prior to the sampling time (2011)

while in the B site it occurred five years before the sampling time (2008), thus potentially masking any difference that might have been expected in these layers. The organic matter is exposed during the replanting due to the disruption of aggregates and this facilitates the mineralisation of soil C resulting in losses (PAUSTIAN et al., 1997; SIX et al., 2002). In the conventional tillage up to 80% of C that potentially was stocked in the first 0-20 cm depth under the adoption of unburned system could be lost during the soil disturbance operation (SILVA-OLAYA et al., 2013). The result of Galdos et al. (2009a) agree with this, a significant difference was found mainly in the topsoil layer (0-10 cm) in the area with 8 years without tillage plus unburned system but it was not significant for other areas with recent soil disturbance. In addition, Blair (2000) also showed higher contents of C might be found in topsoil layers for burned system especially due to the presence of charcoal and ash on the soil surface.

The tillage should occur more often under the unburned system as there is a tendency for soil compaction and consequently lower yields with the intensity traffic of heavy machinery in the harvest system (WALTER et al., 2014). On the other hand, less soil disturbance is strongly recommended since straw retention has potential to promote soil C accumulation; this can also act to lower consumptions of diesel and synthetic N fertiliser, which would have a positive impact on the GHG emissions balance of ethanol (BORDONAL et al., 2012).

The increase in soil C stock for the UB site from 30 to 60 cm depth may have been the result from straw deposition in the surface of the soil which later is incorporated into the soil mainly through tillage. Furthermore, the high rate of root turnover and/or potential additions of filter cake and other composts on the planting groove (usually around 40-50 cm depth) may also lead to higher soil C stock at this depth interval. No difference found in topsoil layers should be due to the recent soil disturbance as mentioned before. In this system, it is expected that about 10 to 20 Mg ha⁻¹ of straw will be maintained on the soil surface, thus this practise promotes higher SOM accumulation in the unburned relative to the burned system (THORBURN et al., 2001). Consequently, the unburned system often has higher fertility (CORREIA; ALLEONI, 2011) than burned system. The organic matter accumulation associated with these practices such as time since adoption of unburned system plus soil texture (CERRI et al., 2011) and the degree of soil disturbance, may have led to high soil C stock in the unburned system.

Taking into account the soil disturbance on replanting the field, the estimation for C accumulation in this study, after the management change (conducted for a period of 12 years) was modest at 30 cm depth, being approximately $0.2 \text{ Mg C ha}^{-1} \text{ y}^{-1}$. Similar results with a lower C accumulation for the first 20 cm depth were found by Feller (2001) and Cerri et al. (2009). Likewise, as we emphasise here, the authors also pointed out that the replanting operations were taking into consideration in the studies. However, there was evidence of an increase in soil C stock considering the approaches examining different depth layers (30 to 60 cm depth). The difference found in this interval had an increase of $0.75 \text{ Mg C ha}^{-1} \text{ y}^{-1}$ for UB site following the management change from burning, which should not be omitted in GHG balance of ethanol. Although it is typical to only consider values up to 30 cm depth, it is important to take into consideration deeper soil layers since it may have a higher potential to avoid soil C losses to the atmosphere than topsoil layers. Moreover, the phasing out of burned systems will bring other environmental benefits, such as a decrease in emissions of black C and GHG in ethanol production (GALDOS et al., 2013).

It is likely that an increase of SOM and soil C stock, or at least the maintenance of their levels, would lead higher yields (PAN et al., 2009). In this sense, Brazilian sugarcane under an improved UB management could contribute to current debates about food security, energy demands and climate changes (LAL, 2004; GOLDEMBERG et al., 2014). The main barriers found in the new management for the whole profile represent a gap that should be bridged by future knowledge. Particular attention should be given to reducing soil disturbances, as well as the quantity of straw that will have in fact a positive effect on the soil without cause other negative impacts. Additionally, it should also be considered that the remains of straw would be used in the coming years for other purposes such as second generation ethanol (cellulosic ethanol) and an increase production is already expected by this sources (USEPA, 2014). Therefore, the use of minimum or no tillage associated with unburned system plus other practices presented in this study (e.g. vinasse) could be a good solution to maintain or even, achieve higher soil C stocks for both surface and deeper soil layers.

2.4.3 Fractions assessment

It is important to understand how the distribution of the soil organic C is partitioned between different soils fractions. These fractions are typically different particle-sizes and have different degrees of stability within the soil profile, and hence relate to the longevity of soil C stock. This in turn has importance with regards to the management practices used (CHRISTENSEN, 2001) and therefore is considered a guide to assessment of sustainability of the system.

In general, the results were homogeneous with few differences found between the managements systems and the layers assessed. In the case of the B to UB management change, the results indicated a significantly higher C content for UB at the interval depth of 0-20 cm for SCF ($P < 0.05$). It is worthwhile to highlight, however that the texture of the soil was not the key driver in these results, since the sites were similar in terms of soil type (S.I. Table S1). In either of the management changes assessed, the C was more concentrated in the SCF whilst OMF contained the lowest concentration.

Lisboa et al. (2009) highlighted that the time of C residence in the SCF, which may be more sensitive to the management used, is higher than any other fraction. On the other hand, OMF represents faster turnover and nutrient cycling in the soil (GAMA-RODRIGUES; GAMA-RODRIGUES, 2008) due to its role for biologic activity. This fraction is usually free (i.e. not linked with mineral matter), and plant and animal residues in various stage of decomposition are common compounds found. Hence, it can often show high spatial and seasonal variability relating to organic residues inputs (CHRISTENSEN, 1992). Even so, in our study no differences after the management changes were noted in any of the cases (B vs UB and NV vs V) assessed and layers for OMF. The likely explanation for this result may be related to faster decomposition of organic material present in the vinasse as well as its higher potential for the decomposition of SOM present at straw, especially provided by higher rates of N in the vinasse (OLIVEIRA et al., 2013). The recent soil disturbance as discussed in previous section may also influenced the soil C stock and no significant difference was found, especially in the case of the B to UB management change. Six et al. (1999) emphasised that soil disturbance might alters the aggregate dynamics, increasing the turnover time of SOM and on the other hand, decreasing the formation of stabilized C fractions. The results of Signor et al. (2014) pointing out lower C content in OMF in sugarcane areas under recent soil disturbance whereas

areas under less disturbance showed higher C content. Our results agree with the results found by Signor et al. (2014), indicating a somewhat higher C content at OMF and SF for B site in relation to UB, where the first did not have soil disturbance in the last 5 years (Figure 7).

On the other hand, OMF is regarded as the main driver responsible for the stabilisation of the macro-aggregates on the soil and as well as has an important role on the changes of the total C content (TISDALL; OADES, 1982). In this way, taking into account those results found of Signor et al. (2014), we suggest that a likely increase of C at OMF prior the soil disturbance in our study areas might have contributed to the higher C found in the SCF for 0-20 cm depth in the UB site. OMF has the ability to promote stabilisation of the C on the soil and the greatest amount of C found at SCF is derived from decomposition processes occurring in the OMF (ROSCOE et al., 2001). In addition, the fact that the B site has the presence of charcoal and ash on the soil surface, might have influenced the lack of difference in the C content in the OM and SF between B and UB system (BLAIR, 2000; ROSCOE et al., 2001).

The V site also showed a slight increase in SCF in relation to NV, nonetheless there was no significant difference between the fractions and layers assessed ($P < 0.05$). Even so, there was a tendency of increases of 20% of C content in the 0-20 cm depth at SCF under V site management after the management change. This result can be also explained due to recent soil disturbance at both V and NV sites as well as because of the small sample size and greater error associated with the vinasse application (Figure 6). Although the vinasse application quantity was equal in all areas, some plots could receive different amount than others causing this error variation.

In general, fractions assessment agree with results found for soil C stock and showed that it could be a potential indicator for response in the management change. Despite the demonstrated linkage, it is necessary a larger sample size to assess the soil C changes at the fractions accurately. Besides that, soil disturbance has seems to have had a direct influence on the C in the soil fractions, especially on the OMF. It is possible to conclude that the management change for UB and V system may contribute to the C accumulation especially on topsoil layers and SCF,

which exhibit C at more stable forms. The potential "trapping" of organic compounds within the stable fractions and the stabilisation by reactions with the minerals surface (complexation) are important mechanisms for maintaining the higher organic matter levels in the soil (CHRISTENSEN, 2001).

2.4.4 Modelling assessments

The modelling prediction supported the hypothesis that the changes in sugarcane management to improved practices in the field could increase soil C stocks also, in the top of 20 cm of depth.

As discussed before, it is important to stress that the Century model was used as a tool to conduct the modelling approaches, up to the equilibrium state of soil C stock. The model simulations performed showed higher soil C stock in natural vegetation compared to any other management assessed as expected and they were well-defined by measured results (MELLO et al., 2014). Besides that, the results highlight the significant soil C losses after LUC from natural vegetation to pasture which was also found by Mello et al. (2014) and modelled by Silva-Olaya (2014).

After the introduction of the improved sugarcane practices an increase in soil C stock was noted in the long-term simulations (Figure 9). This was more evident in the management change from burned to unburned system. Although the observed differences between the burned and unburned area in 2013 were not significant differences, the unburned area had an increase in soil C stock of almost 3% per year from 2001 to 2013. In this same period modelling assessments suggested that, the gains in the unburned site were $0.75 \text{ Mg C ha}^{-1} \text{ y}^{-1}$ while the burned site had a C depletion of $-0.50 \text{ Mg C ha}^{-1} \text{ y}^{-1}$.

The difference between the management systems tends to decrease whereas the steady-state is achieved in simulated long-term approaches. By the year 2049, considering a projected period of 35 years, the increase of soil C stock from the B to U system were approximately $0.5 \text{ Mg C ha}^{-1} \text{ y}^{-1}$, compared to an increase of only $\sim 0.06 \text{ Mg C ha}^{-1} \text{ y}^{-1}$ when the equilibrium state was achieved. After this period, there is a likely pattern tends of the continuous lower increase under the UB system after 2050 and a decrease under the B system (Figure 9b), but in much smaller proportions. These results agree with measured values found by Razafimbelo et al. (2006) who reported an increase rate of $0.65 \text{ Mg ha}^{-1} \text{ y}^{-1}$ in the topsoil layer (10 cm) under an unburned area after 6 years of its use (one cycle). Although, the authors

suggested further research in order to assess the effects of tillage disturbance on replanting sugarcane. Our simulated long-term approach highlights that the increase in rates of soil C stocks may be similar for 0-20 cm depth taking into consideration tillage each 6 years and a period of 35 years, i.e. six tillage.

Owing of the lack information under pasture management, we assumed a typical management practice in our simulations, taking into consideration that several studies have reported the degradation of the pastures in Brazil (BATLLE-BAYER et al., 2010). In agreement with our simulations, Maia et al. (2009) also found similar rate of losses of C in degraded pastures ($-0.28 \text{ Mg C ha}^{-1} \text{ y}^{-1}$) previously covered by Amazon forest and Cerrado vegetation. Silva-Olaya (2014) pointed out that LUC in areas with sugarcane following pastures resulted in losses of $-0.19 \text{ Mg C ha}^{-1} \text{ y}^{-1}$. In relation to LUC for cropland, the annual rate of soil C losses found in our study is in accordance with earlier studies (CARVALHO et al., 2010; SILVA-OLAYA, 2014).

In turn, the transition to NV and V system on the sugarcane field followed from pasture, and it had been this land use for 10 years in both sites after the removal of the native vegetation. At first the model indicated the same trends of soil C stock however it had changes after the application of vinasse under the V system. From 2004 (first year of vinasse application) to 2013, the V system had an increase of $0.44 \text{ Mg C ha}^{-1} \text{ y}^{-1}$ (similar to the measured results $0.55 \text{ Mg C ha}^{-1} \text{ y}^{-1}$ at 0-30 cm) whereas the NV site had an increase of only $0.23 \text{ Mg C ha}^{-1} \text{ y}^{-1}$ in the same period. This lower increase demonstrated under the NV system can be explained by the use of unburned in the site through this period (from 2009) since the increase prior to this year was not observed.

There are not many studies relating whether the effect of vinasse in the long-term will result in a positive effect on soil C stocks. In our simulated long-term approaches, the differences on soil C stocks between the system/scenarios tended to decrease when equilibrium was reached. In this way we conclude that, the use of unburned practices in both systems should be the responsible for the lower differences in long-term evaluation and is the key factor responsible for the increase on soil C stock. Despite this, it is notable that in the first 19 years (2009-2028) even in both sites under unburned management, the use of vinasse showed a high potential to increase soil C stock. The greatest difference between the systems was found exactly in the year of 2028 when the potential equilibrium state was achieved for both cases (Figure 9b). Similar measured results and differences were detected

at topsoil layers (0-20 cm) by de Resende et al. (2006), studying 16 years of vinasse plus unburned management, with an increase in the rate of around 4 Mg C ha⁻¹, found in the same study. Zolin et al. (2011) also reported differences with higher C content under vinasse application area after 20 years, however the authors did not mention whether the areas examined were under burned or unburned management. Brandani et al. (2014) reported similar results by simulation approaches in a site with unburned plus additional organic amendments such as vinasse and filter cake.

The use of vinasse, especially under burned management, could be used under any doses to avoid a high decline in soil C stocks (Figure 10a). Soil C stock was 30% lower than native vegetation under only burned system, so that after vinasse application the difference on soil C stocks decreased to 20% at high dosages in relation to the native vegetation by the year 2150.

Likewise, the use of vinasse in an unburned system showed similar dynamics to the burned system. However, in this case, higher soil C stocks were found to exceed those of native vegetation and under the scenario of unburned without vinasse (Figure 10b). At standard doses of vinasse application (those applied usually by the mill), an increase of 20% was observed whereas lower and higher levels had an increase of 19 and 22%, respectively compared to native vegetation.

While vinasse application may have clear benefits to increase soil C stocks, this is not the only issue which needs to be considered; salinisation and GHG emission are also important. Fuess et al. (2014) reported negative effects due to the unrestricted use of vinasse, and also highlighted to other alternatives of its reuse (biogas, among others).

To summarise, our modelling approaches and empirical findings in this study have considered the key management changes and, importantly, have examined these transitions in the same location. In addition, the changes in sugarcane management to improved practices assessed here may have an important key role in order to reduce the payback time for soil C stock and sugarcane ethanol as indicated by Mello et al. (2014). It was more evident on cessation of burning where the C had increased by almost 40% in simulate long-term. In addition, vinasse also showed positive effects on soil C stock, with the greatest impact on topsoil layers. The use of vinasse under burned systems could be the most readily available first step to reduce sharp decreases in soil C stocks usually found under burned systems.

In this regard, the results also must be taken into consideration when it comes to the carbon footprint of ethanol. Based mostly on the forecast expansion of 6.4 Mha of sugarcane up to 2021 reported by Goldemberg et al. (2014) to meet the future ethanol demands and consequently the huge amount of vinasse produced, both these practices are important for the sustainable expansion of sugarcane production as well as to meet future environmental targets.

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SUPPORTING INFORMATION

Table S1. Mass soil in each of SOM of the areas cultivated with sugarcane in Ourinhos (SP) under different management practices (NV) No vinasse; (V) Vinasse; (B) Burning; (UB) Unburned.

Reference area	Depth cm	OMF	SF	SCF	Mass recovery
		(>0.053 mm)	(>0.053 mm)	(<0.053 mm)	
----- g dry soil ⁻¹ -----					
NV	0-10	0.09	3.68	16.18	19.95
NV	0-10	0.07	1.72	18.16	19.95
NV	0-10	0.11	2.54	17.09	19.74
NV	10-20	0.06	2.79	17.08	19.93
NV	10-20	0.01	3.01	16.78	19.80
NV	10-20	0.02	5.21	14.09	19.32
NV	40-50	0.38	8.22	10.87	19.47
NV	90-100	0.16	9.66	9.97	19.79
V	0-10	1.04	4.80	13.33	19.17
V	0-10	0.05	4.65	15.09	19.79
V	0-10	0.11	8.94	10.89	19.94
V	10-20	0.02	4.02	15.92	19.96
V	10-20	0.02	4.65	14.80	19.47
V	10-20	0.07	10.24	9.67	19.98
V	40-50	0.54	11.14	8.03	19.71
V	90-100	0.18	10.09	9.47	19.74
B	0-10	0.20	5.22	14.50	19.92
B	0-10	0.09	4.59	14.91	19.59
B	0-10	0.14	7.11	12.27	19.52
B	10-20	0.11	3.24	16.52	19.87
B	10-20	0.08	4.45	15.41	19.94
B	10-20	0.10	7.49	12.38	19.97
B	40-50	0.01	10.71	9.02	19.74
B	90-100	0.01	11.54	8.41	19.96

UB	0-10	0.09	4.49	15.28	19.86
UB	0-10	0.14	4.34	15.39	19.87
UB	0-10	0.01	4.38	15.31	19.70
UB	10-20	0.03	4.86	15.09	19.98
UB	10-20	0.08	5.78	14.01	19.87
UB	10-20	0.01	2.76	16.89	19.66
UB	40-50	0.02	9.95	9.53	19.50
UB	90-100	0.01	10.82	9.00	19.83

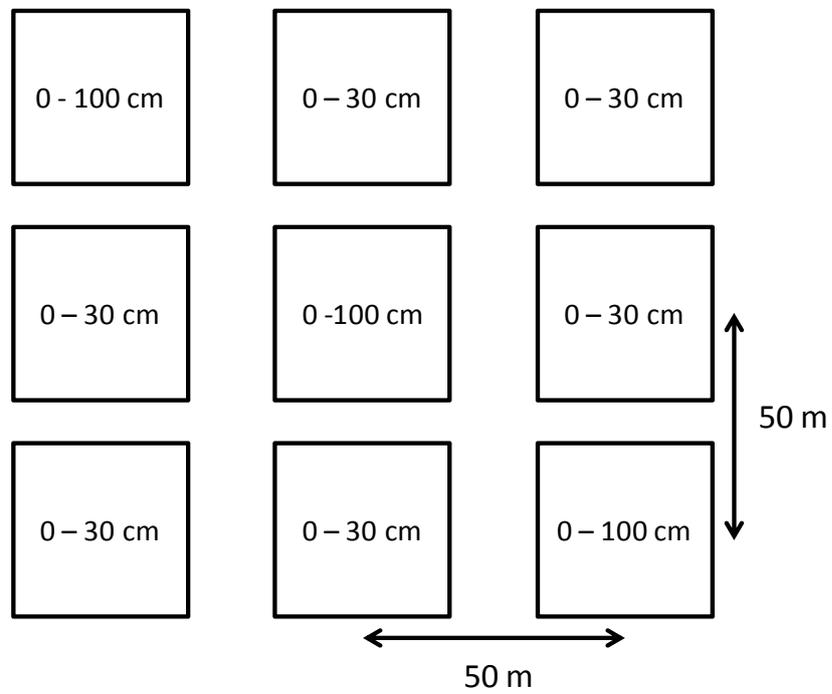


Figure S1. Experimental design of the soil sampling adapted from Mello et al. 2014.

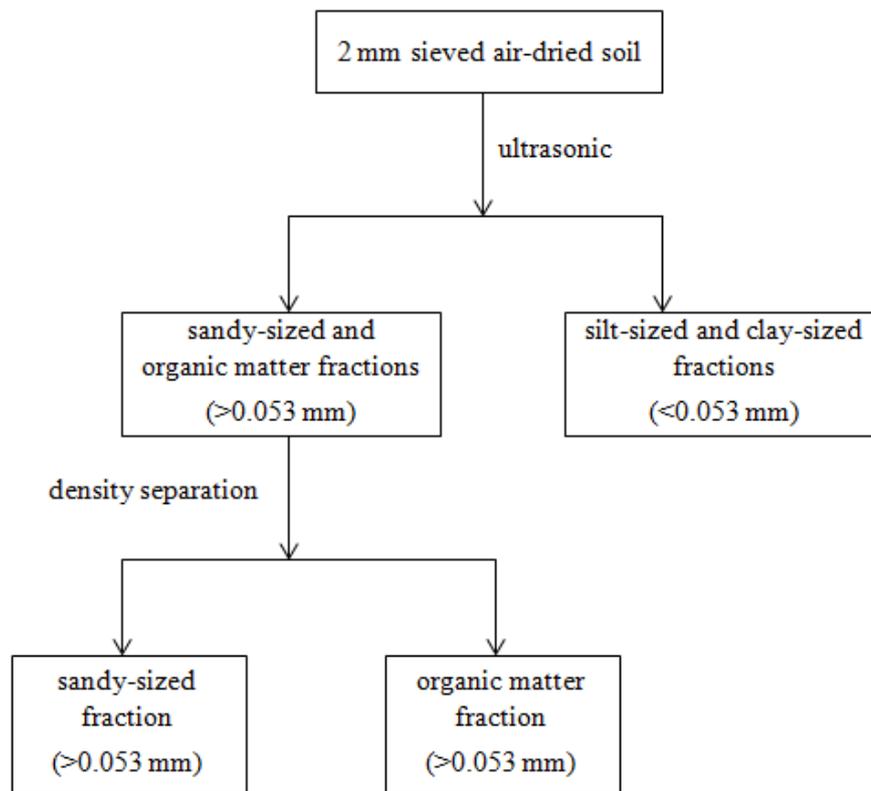


Figure S2. Scheme of the stages of the soil physical fractionation.

3 Impacts on soil carbon stocks of direct land use change from semi-arid woodland to irrigated biofuel crop in Brazil

Abstract

Given the growing population and increasing land use demand for energy supply and food productions, governments are looking for alternative solutions which could provide with higher yields and low carbon (C) potential. Sugarcane as a bioenergy crop has been appointed as precursor to substitute fossil fuels through ethanol production. Ethanol production might provide further mitigation through increased soil C accumulation in sugarcane fields, and reduced greenhouse gas (GHG) emissions. However, to meet the demand for sugarcane expansion, it will be necessary to promote sugarcane expansion in areas where the climate to grow the crop is not considered suitable. These areas, such as Brazilian semi-arid regions, will require full or supplementary irrigation water, even though the impacts of irrigation on soil C stock are not fully understood. The aim of this study was to evaluate the soil C stock after land use change (LUC) from dry-land native vegetation to irrigated sugarcane system. The chronosequence analysed consisted of three areas: native vegetation dry-land, sugarcane for 4 years (I4) and 6 years (I6) in a semi-arid region in Brazil. Additionally, the CENTURY model was used to evaluate the long-term impact of this management approach. The use of the irrigation system resulted in a slight increase in soil C stock for topsoil layers. It was, however, less prominent than expected, perhaps due to relatively few years since conversion. Measured values of soil C stock and C content of different particle size fractions did not significantly differ between native vegetation dry-land and irrigation sugarcane fields. Nevertheless, data suggest that irrigation for a greater number of years may increase soil C stock in topsoil layers (0-20 cm). On the other hand, irrigation system likely could result in losses for deeper layers. Simulated long-term modelling showed an increase in soil C stock of $0.3 \text{ Mg ha}^{-1} \text{ y}^{-1}$ during at least 40 years for 0-20 cm depth. Future studies are required to assess the mechanisms by which irrigation can impact soil C stocks and longer-term effects of irrigation on both C budgets and other environmental impacts.

3.1. Introduction

Global population has increased rapidly over the last century and is projected to increase by another 40% by 2050 (UNITED NATIONS, 2014), resulting in increased demands on agriculture and energy production. A huge net flux of carbon (C) has been released to the atmosphere, especially in the last two centuries, due to the expansion of cultivated agricultural land area, which has led to decreased C stocks in soils globally (HOUGHTON et al., 1983). Governments are actively seeking for low C solutions to energy and food production to meet demand from a growing population. In Brazil, sugarcane production has great potential as a source of energy (ethanol) and food (sugar). As an energy source, sugarcane could contribute as renewable alternative to substitute fossil fuels.

There are approximately 20 million hectares cultivated under sugarcane worldwide. Brazil is a leading producer with substantial quantities in productivity and in land area, with around 10 Mha of land intended for sugarcane production (FAO, 2015). Even so, to meet projected future demand requirements by 2021, expansion in both land area used for the crop, as well as productivity, is required (GOLDEMBERG et al., 2014). The Brazilian government has been looking for strategies to promote sugarcane expansion in areas where the climate is considered sub-optimal, as well as promoting an improvement in yields in order to achieve the levels of biofuel production required (MANZATTO et al., 2009). Based on Renewable Energy Sources Directive (RES-D) such expansion may not occur from native vegetation or high biodiversity grassland. Since Brazil has other potential lands under other uses for this regard the main recent expansion to sugarcane production has been occurred from pasture (ADAMI et al., 2012). Sugarcane is well-established in the centre-south Brazil, however in some areas in the north-eastern region (Brazilian semi-arid region) the ability to support sugarcane production is currently limited due to low rainfall. The Brazilian semi-arid region covers 980000 km², which is 11% of the total of national territory (MEDEIROS et al., 2012), and the main issue for growing crops in this region is the limitation of water availability.

A new sugarcane management technique has been applied in this region of Brazil, promoting the irrigation of sugarcane fields. According to the National Water Agency (ANA), 3.8 million hectares of Brazil has sugarcane irrigation, being headed by a project called "Projeto Jaiba". This aims to improve irrigation technologies in order to boost production of food and energy crops in these regions.

This is located in the North of Minas Gerais state in the semi-arid region (Caatinga biome) and has led to a socio-economic boom for the region. The replacement of other land uses as well as the native vegetation and the use of irrigation, however, might have several consequences for the soil system.

It is predicted that increased yields, due to irrigation practices, would lead to increased input and accumulation of soil organic carbon (COLLINS et al., 1992; FOLLETT, 2001). The drivers of these increases are enhanced physical protection of C within soil aggregates and higher soil organic matter (SOM) inputs (SIX et al., 2004, DENEFF et al., 2008). While higher yields have been reported for irrigated sugarcane (WACLAWOVSKY et al., 2010; MONTEIRO; SENTELHAS, 2014), evaluations of soil C stock changes in irrigated sugarcane systems have not been found in the literature. C sequestration rates have, nevertheless, been estimated using plant C inputs expected under irrigation system, and an increase between 0.05 to 0.15 Mg C ha⁻¹ y⁻¹ was pointed out (LAL et al., 1998). It is important to highlight that there is an extensive discussion about a maximum potential for soil C storage, or C saturation, in addition to other impacts which may be caused by irrigation system.

An irrigated system results in more favourable environmental conditions for microbial activity, which in turn can lead to higher C mineralisation and limiting the expected increase in soil C stocks (SHIPPER et al., 2012; GIUBERGIA et al., 2013). In droughted regions, this process has been highlighted as being responsible for observed decreases in soil C stocks (CHURCHMAN; TATE 1986; CONDRON et al., 2014). Gillabel et al. (2007) and Giubergia et al. (2013) have found that potential changes on soil physical structure, as a result of irrigation, may also impact negatively on aggregate resistance.

Ultimately soil C stocks under irrigated systems will probably depend of the balance between increased C inputs through higher yields and increased outputs related to increased microbial activity and changes in physical soil structure. There is also a question of the sustainability of intensively irrigated agriculture, due to changes in water availability, as a result of increasing population and climate change in the future. The impacts of irrigation on soil C stocks is not fully understood, thus a full assessment is essential in order to understand an important component of the system.

The aim of this study was to evaluate soil C stock (to 1 metre depth) under a chronosequence of sugarcane cultivation as influenced by irrigation and to use models to assess the long term impacts of this practice on soil C stock and compare measured findings to modelled outcomes. The C content of particle-size fractions was also evaluated in order to assess the qualitative aspects of C storage and the relationship between irrigation systems with the distribution of C in different particle-size classes after land use change (LUC). We hypothesized that i) irrigation will increase soil C stock in the top soil layers, relative to native dry-land vegetation, due to the higher plant-derived to soil, and ii) irrigation will lead to a decrease in soil C stock in deep layers, likely due to enhanced microbial activity and potential leaching.

3.2. Material and methods

3.2.1 Description of the study areas

The study areas were located at SADA Bioenergia e Agricultura in Jaíba, Minas Gerais state (15.12°S , 43.56°W , 478 m a.s.l), which represents the extreme north of the Southeast region in Brazil (Figure 1).

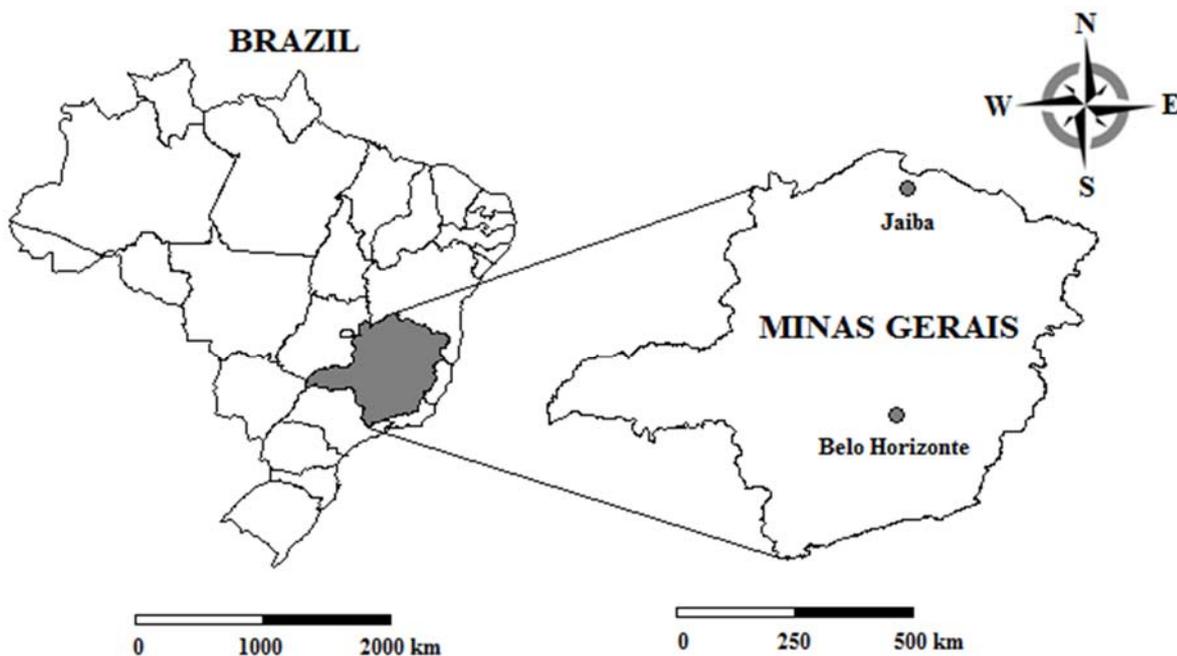


Figure 1 - Location of the study area in Brazil and Minas Gerais state. Belo Horizonte city is the Capital of Minas Gerais state

The region represents a semi-arid land in Brazil where irrigation practices are a common management used in the most of the agro-ecosystem. The climate region is classified as BSh according to Köppen classification, characterized by hot and dry seasons, typically dry winter and rainy summer, being at least 10 dry months during the year (PRADO, 2003). The mean annual precipitation is 800 mm concentrated in the spring-summer months from November to January while the annual average for minimum and maximum temperature is 18°C and 31°C, respectively (Figure 2).

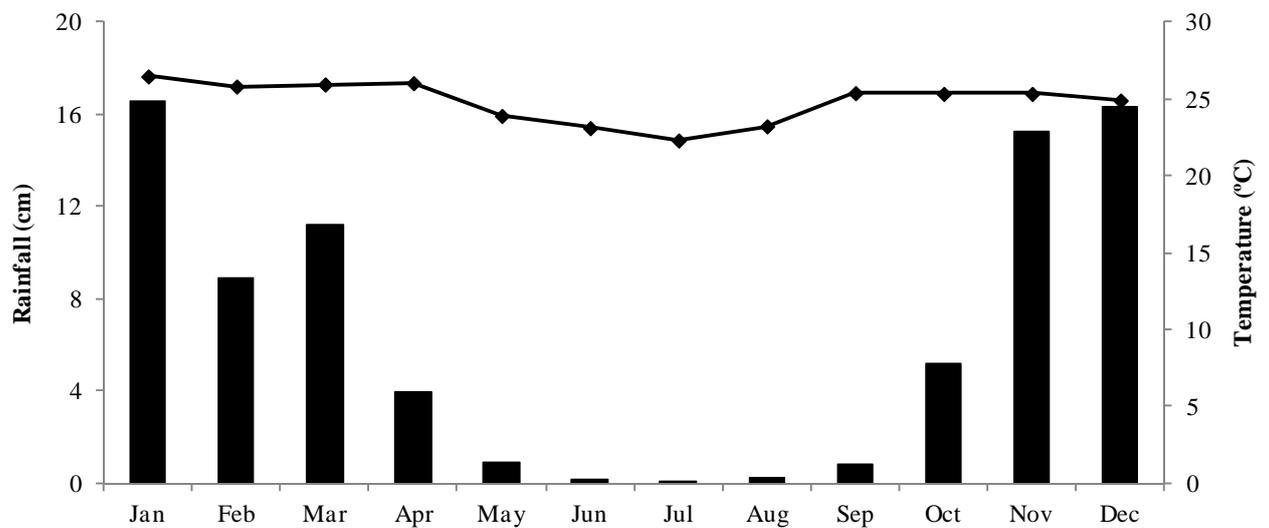


Figure 2. Average monthly rainfall and temperature distribution in the region of Jaíba-MG between 1977 to 2013.

These values represent an average of 36 years recorded weather (1977-2013) from the Instituto Nacional de Meteorologia (INMET) stations located in Janaúba-MG and Mocambinho-MG (69 and 190 km far from the areas, respectively) and for last 5 years from the SADA Bioenergia e Agricultura (agro-climatic station closer to study areas).

The native vegetation of the region is regarded as a transitional area (SCOLFORO et al., 2008) between woody Caatinga woodland and Cerrado usually found across whole northeast of Brazil and in a part of northern Minas Gerais state. The predominant vegetation is typically composed by xerophytic trees and shrubs (RIZZINI, 1979, 1997). Botanical families' dominance are Mimosaceae, Caesalpinaceae, Fabaceae and Bignoniaceae (DEL REY, 1991).

Jaíba is characterized for having a greater range of agro-ecosystems under irrigation and recently the water has been applied also in sugarcane production. The chronosequence approach was used to examine changes in soil C stock due to LUC (native vegetation to sugarcane). This provides the possibility to study the temporal dynamics of soil C sampling at one time point instead of sampling across multiple years, with a space-for-time substitution. The chronosequence areas need to have identical soil type, climatic conditions and similar land use history since the occurrence of LUC. One chronosequence series (3 areas) was identified that fulfilled all the above criteria; a stand of non-irrigated native vegetation (NV) was sampled adjacent of the sugarcane areas as a reference and the irrigated areas had been planted with sugarcane for four (I4) and six (I6) years. The distance of the areas within the chronosequence had less than 5 km.

The soil was classified as Arenosol (FAO, 2014), Entisol -Quartzipsamments (SOIL SURVEY STAFF, 2014), and Neossolo Quartzarênico (Brazilian Classification Soil System, SANTOS et al., 2013) and soil properties are shown in the Table 1. This soil is characterized by low fertility and low water retention capacity as well as moderately acid and with small amounts of SOM contents.

Table 1 - Soil textural properties and bulk density under sugarcane irrigation areas for four years (I4), six year area (I6) and under native vegetation (NV), up to 1 metre depth

Reference area	Depth cm	Sand	Silt		Clay	Texture (SBCS)	BD g cm ⁻³
			g kg ⁻¹				
Native vegetation (NV)	0-10	892	33	74	sandy	1.48 ± 0.00	
	10-20	899	24	77	sandy	1.53 ± 0.00	
	20-30	880	22	98	sandy	1.49 ± 0.00	
	30-40	842	32	127	sandy	1.48 ± 0.02	
	40-50	842	22	136	sandy	1.53 ± 0.01	
	50-60	825	24	151	medium	1.52 ± 0.02	
	60-70	805	43	152	medium	1.53 ± 0.02	
	70-80	790	22	189	medium	1.56 ± 0.02	
	80-90	783	40	177	medium	1.53 ± 0.02	
	90-100	779	44	177	medium	1.56 ± 0.02	
Irrigation 4 years (I4)	0-10	859	20	121	sandy	1.47 ± 0.00	
	10-20	881	21	98	sandy	1.54 ± 0.00	
	20-30	879	23	98	sandy	1.57 ± 0.00	
	30-40	881	20	100	sandy	1.59 ± 0.02	
	40-50	879	20	101	sandy	1.57 ± 0.02	
	50-60	866	22	112	sandy	1.50 ± 0.02	
	60-70	853	47	101	sandy	1.48 ± 0.03	
	70-80	860	43	98	sandy	1.48 ± 0.01	
	80-90	837	13	150	medium	1.45 ± 0.01	
	90-100	836	15	148	medium	1.45 ± 0.02	
Irrigation 6 years (I6)	0-10	886	13	101	sandy	1.49 ± 0.01	
	10-20	883	19	97	sandy	1.61 ± 0.00	
	20-30	861	13	126	sandy	1.68 ± 0.00	
	30-40	847	28	125	sandy	1.62 ± 0.03	
	40-50	842	33	125	sandy	1.55 ± 0.01	
	50-60	833	18	149	medium	1.5 ± 0.02	
	60-70	830	20	149	medium	1.51 ± 0.02	
	70-80	833	19	149	medium	1.47 ± 0.01	
	80-90	829	23	148	medium	1.45 ± 0.03	
	90-100	812	24	164	medium	1.46 ± 0.02	

SBCS: Brazilian soil classification system.

Irrigation is achieved using a central pivot system (Valley model 4865-8000) which has a speedometer of 264 m h⁻¹ and output of 246 m³ h⁻¹, 3.92 mm per turn. The water was taken up from Distrito de Irrigação de Jaíba (DIJ) canals, from the São Francisco River. The irrigation rate in the sugarcane areas (I4 and I6) is variable and based on weather forecasts and soil moisture tests. Irrespective of the soil type, in the sugarcane production, irrigation is usually done throughout at least 10 months per year. The mean rate applied for the areas ranged from 800 to 1000 mm of irrigation water regularly distributed between the months of February to November.

I4 or I6 had direct LUC from native vegetation to sugarcane. Both areas have had mechanized harvesting since the conversion and conventional tillage plus mineral fertiliser application. However, it is important to stress out that none of the sampled areas had tillage after planting (2005 and 2007). Likewise, all sampled areas had the same nutrients application: nitrogen (N), phosphorus (P) and potassium (K) in the plant stage (600 kg ha⁻¹ 6-30-24), N and P were also applied annually by ferti-irrigation (100 kg ha⁻¹) while calcareous was applied only in I6 area at a rate of 5 Mg ha⁻¹ (only in the sugarcane plant stage). The histories of each one of these sites, including the management practices, are described in Table 2.

Table 2 - Summary of period of time and brief description about the history in each study area

Management detailed	Sugarcane I4	Sugarcane I6
Period with sugarcane field (years)	4	6
Period of under each management system (years)		
No Burn and manual harvesting	4	6
Irrigation practice	4	6
Vinasse application	-	-
Organic fertilisation	-	-
Mineral fertilisation	4	6
Amount of water applied (mm)	~ 800/1000	~ 800/1000
Timeline before sugarcane field		
Years of deforestation	2007	2005

3.2.2 Approach, sampling method and analyses

The experimental design in each area followed the schematic suggested by Cerri et al. (2013) and utilized by Mello et al. (2014) (Figure S1), but with the exception that, more layers were sampled in this study. The sampling site covered approximately 1 ha (100 × 100 m) and consisted of nine trenches distributed in 3 × 3 grid spaced at 50 m apart. Three deeper sampling trenches were sampled in 10 cm increments to 1 m (deeper trenches 120 × 120 × 120 cm). In each of the three deeper trenches, soil samples were taken from two different soil walls to encompass between and within plant rows. The other six trenches were sampled to 30 cm depth (mini trenches 40 × 40 × 40 cm) with sampling being from one wall only in 10 cm increments. In each layer of each trench (deep and mini), two types of samples were taken: undisturbed samples were taken for bulk density assessments using stainless steel rings with a diameter and height of 5 cm (98.17 cm³ internal ring volume) (BLAKE; HARTGE, 1986) and also for determining the C and N content (78 samples), and disturbed samples were taken for chemical and physical analyses (60 samples). All soil samples were taken in August 2013 with a total of 138 samples for each of three study sites.

3.2.3 Soil analyses

The fresh mass for each sample was recorded in the laboratory. All samples were air-dried and sieved with a 2 mm mesh in order to remove stones and coarse roots fragments prior to soil analyses. Disturbed samples taken from deeper trenches were used to determine contents of sand, clay and silt, as well as pH (CaCl₂) and the concentration of macro and micronutrients, according to EMBRAPA (1997) and Anderson and Ingram (1989). All of the layers (from 0 to 100 cm) were used for physical analyses (three replicates). While only the following depths (0-10, 10-20, 20-30, 40-50 and 90-100 cm) were used for chemical analyses (one sample consisting of three deeper trenches).

Soil moisture of all samples was determined by drying soil at 105° C for 12 hours followed by reweighing. To determine the total C and N only the undisturbed samples were sieved through 100 mesh (0.150 mm) and ground to a fine powder prior to analysis. The total soil C and N were determined by dry combustion (NELSON; SOMMERS, 1996), using a Leco Truemac CN elementary analyzer

(furnace at 1350° in pure oxygen). The physical and chemical soil attributes is presenting in Tables 1, 3.

Table 3 - Soil chemical analyses under irrigation sugarcane areas and native vegetation

Reference area	Depth cm	pH	P mg dm ⁻³	K	Ca	Mg	H+Al	SB	CEC	BS %
Native vegetation (NV)	0-10	6.7	34	1.3	42	3	10	46	56	83
	10-20	4.9	3	0.6	6	0.1	16	8.0	24	31
	20-30	3.9	2	0.4	1	0.1	16	2.0	19	12
	40-50	3.9	2	0.4	0.1	0.1	28	2.0	30	5.0
	90-100	3.9	1	0.8	0.1	0.1	22	2.0	24	9.0
Irrigation 4 years (I4)	0-10	5.8	29	3.8	21	11	13	36	49	73
	10-20	5.2	9	2.7	9	3	11	14	25	57
	20-30	4.9	3	1	6	3	15	10	25	41
	40-50	3.9	2	0.4	0.1	0.1	16	2.0	18	10
	90-100	3.8	1	0.4	1	0.1	25	2.0	27	8.0
Irrigation 6 years (I6)	0-10	5.8	12	1.3	18	8	13	27	40	67
	10-20	5.7	5	1	10	5	13	16	29	54
	20-30	5.8	2	0.6	9	4	10	13	23	58
	40-50	4	1	0.4	1	1	22	3.0	25	11
	90-100	4	1	0.5	21	11	18	32	51	64

SB=sum of bases, CEC=cation exchange capacity, BS=base Saturation

3.2.4 Physical fractionation

LUC can affect the soil C distribution within each particle sizes of soil, thus physical fractionation of the soil is a valuable technique in order to assess qualitative C after such changes and the potential redistribution (CHRISTENSEN, 2001). Physical fractionation was made according to Christensen (1985, 1992), using a method which separates the particles sizes by dispersion, wet sieving and sedimentation.

The physical fractionation was carried out for the following depths: 0-10 cm and 10-20 cm in triplicate, and 40-50 cm, and 90-100 cm for all studied areas. For this 20 g of dry soil was sonicated in 60 ml of deionised water for 15 minutes. Subsequently, the sample was passed through a sieve of 270 mesh (0.053 mm) to separate the particle size fractions of the soil (Figure S2). The free organic matter (OMF) and sandy fractions (SF) both >0.053 mm, remained in the sieve which were separated by the density separation method utilising only distilled water. Silt and clay fractions (SCF) <0.053 mm, passed through the sieve and were quantified together. All fractions were dried, weighed, and the total C content determined using the same procedures described in the “Soil analyses” section.

3.2.5 Soil C stock

Soil C stock was calculated by multiplying the soil C concentration (%) and bulk density (g cm^{-3}) for each 10 cm layer. As soil C stock is directly related to bulk density which can be altered by different LUs, it was necessary to adjust the mass of soil layers being compared to a reference mass according to Ellert and Bettany (1995). Bulk density data from native dry-land vegetation was used as reference for sugarcane areas in order to derive the equivalent soil mass across each land use.

3.2.6 Century model description

Century model version 4.0 (PARTON et al., 1987) was used to simulate the main long-term changes in soil C stock for the upper 20 cm of the soil profile. Though the original model has been developed for prairies in North America it has been used widely for annual and perennial crops, forest and pastures (PARTON; RASMUSSEN, 1994; CERRI et al., 2004; 2007; CONG et al., 2014). The models has also shown to be reliable results for sugarcane simulations and has a range of options for simulating management systems (KEATING et al., 1994; VALLIS et al., 1996; GALDOS et al., 2009; 2010; BRANDANI et al., 2014).

Parton et al. (1987) described a detailed description of the structure of the model. In summary, the Century model is separated in several sub-models. Litter is separated into two fractions (metabolic and structural), and there are three soil organic matter pools (active, passive and slow), with each one having a different decomposition rate. Above and belowground litter pools and surface microbial pool

are directly linked to the decomposition of surface litter. Different plant production submodels (grassland, crop and forest systems) are directly associated with at same SOM and nutrient cycling submodel (METHERELL et al., 1993).

3.2.7 Initialisation, parameterisation and assessment of the model

The initialisation of the model requires site-specific parameters including soil texture, bulk density, pH and N inputs (atmospheric and biological). Weather data requirements include mean precipitation and minimum and maximum monthly temperatures with a long historical period. In view of this, the simulations were run using monthly meteorological data collected for period of 36 years of recorded weather (1977-2013) from a compilation of three stations: INMET (Janaúba and Mocambinho city and SADA mill (near to the study areas).

The “site.100” file used site parameters data from NV area in order to initialise the first pre-cultivation conditions in a Century forest sub-model. In order to initialise the first pre-cultivation conditions, the model was run to equilibrium state (7000 years) under Caatinga native vegetation until 2005 at the I6 area and until 2007 at the I4 area. The main focus of this study was not to make changes in the model; however some adjustments were required due to native vegetation is located in a transitional situation. Model parameterisation for native vegetation was made adjusting the photosynthetic efficiency parameter ($PRDX=700 \text{ g dry matter m}^{-2}$), using an average between data from several studies carried out in the semi-arid regions and Caatinga Biome (KAUFFMAN et al., 1993; SCOLFORO et al., 2008; BAILIS, MCCARTHY, 2011; MENEZES et al., 2012). The monthly production of C was assumed as a percentage of 45% of the maximum gross forest production. Parameters in relation to lignin content, maximum leaf index for mature forest and the monthly death rate for large wood and coarse was adjusted based on data reported by Althoff (2010). The other values from Caatinga vegetation, specified by Century 4.0, were used for all the remaining parameters.

Subsequent checks were performed to ensure the simulated above and belowground biomass was in line with measured data from published studies (SCOLFORO et al., 2008; BAILIS; MCCARTHY, 2011). Bailis and McCarthy (2011) estimated C in above and belowground biomass nearby of the present study while Scolforo et al. (2008) accessed a variety of data in the semi-arid region of Brazil.

In addition, it was made a comparison between our simulated results of leaves and roots with measured values reported by Vieira et al. (2009), Menezes et al. (2012) and Aguiar et al. (2014). Finally, it was made validation of the model between simulated soil C stocks with our measured values. At the end of the equilibrium run simulated SOM levels, deforestation process was set up following procedures events parameterised by Cerri et al. (2004) which involve slash and burn.

Afterwards, the model was run with sugarcane parameterisation for each study area based on values developed and reported by Galdos et al. (2009, 2010), which included unburned system (or green harvesting cultivation). Furthermore it was assumed a total amount of 1000 mm y⁻¹ of water application for each sugarcane field based on a history of the mill, and it was divided into 10 months (February to November). Each simulation scenario was informed by land management history from the mill and local farmers as summarised in Table 2.

The accuracy of model simulations to estimate sugarcane growth was estimated by comparing simulated aboveground biomass with yields obtained in the field. It was assumed that the C content on sugarcane plant biomass is 44% (SPAIN; HODGEN, 1994; ROBERTSON, 2003) whereas water content in the mature stalk is 70% (BULL; GLASZIOU, 1975). Finally, correlation coefficient (*r*) and root mean square error (RMSE) were used to compare the simulated and measured soil C stocks, in accordance with Smith et al. (1997).

3.2.8 Long-term scenarios

After evaluation on the model outputs, simulations were extended to predict long-term effects of irrigated sugarcane up to 2100 (87 years), once our transitions are relatively “young”. Each sugarcane cycle was determined with 7 years growth following conventional tillage. In addition, through it is possible to know how long it will potentially take to achieve the equilibrium state or steady state, i.e. maximum accumulation of C. The monthly weather conditions were based on the general average (1977-2013) regardless of the potential climate change, variation in atmospheric CO₂ concentrations or other factors that may influence sugarcane growth and soil respiration.

3.2.9 Statistical analyses

Differences in cumulative soil C stocks between the land uses at 30, 50 and 100 cm were tested using linear models (LM) as was the C content in the soil fractions at 20 cm. Model residuals were inspected for normality.

Differences in soil C stock depth between land uses types were tested using a bootstrap re-sampling and loess regression with R stats package (R CORE TEAM, 2014). For this, all soil C stock values are treated as independent data points. Firstly, the combined land use data (native vegetation and irrigated sugarcane), was re-sampled by bootstrap, with replacement ($n=1000$). These data were then modelled using loess regression and the bootstrap samples used to generate 95% confidence intervals around a modelled soil C profile. This represented the null hypothesis that there was no difference between the land uses types. The data from the new land use only was then modelled using loess regression; if the modelled line for this soil C profile sits outside the confidence interval based on the null hypothesis, it can be inferred that soil C stocks are significantly different.

3.3. Results

3.3.1 Soil carbon stock

There was an overall trend to increase soil C stocks after the replacement of NV to the I4 area, while the I6 area showed only an increase at 0-30 cm depth and a slight decrease across the whole profile (0-100 cm depth). Total cumulative soil C stock for NV system was 22.1 (± 0.2), 33.5 (± 2.2) and 53.2 (± 2.1) Mg ha⁻¹ for the layers 30, 50 and 100 cm, respectively, and for I4 was 25.9 (± 0.3), 34.7 (± 1.7) and 54.6 (± 1.9) and for I6 area was 24.1 (± 0.3), 33.6 (± 3.2), 48.9 (± 3.1) Mg ha⁻¹ for the same respective layers interval. Despite the increase observed in both sugarcane areas, there difference was only significance between NV and I4 for 0-30 cm depth ($P>0.05$).

Looking at only the soil C stock in individual depth increments, similar values were observed for all areas studied. There was a trend of increased soil C stock for the whole profile, top and deeper soil layers, in the I4 area after LUC, whilst a slight decrease was noted in the I6 area from 30 cm to 1 metre depth

in relation to NV dry-land. These observations were confirmed in the loess regression analyses (Figure 3 a, b).

In the topsoil layers, the I6 area, showed the highest values for 0-10 cm depth reaching 18.62 Mg ha^{-1} . However no significant differences were noted between NV area and I6 for this depth. There was only statistical difference (within 95% C.I) in the following depths 20-30 and 30-40 cm with higher soil C stocks for I4 in relation to NV (Figure 3a).

In the subsoil layers (between 70 cm to 1 m depth), I6 area showed the lowest values (mean of 2.8 Mg ha^{-1}) while NV area achieved higher values at this depth interval (mean of 4 Mg ha^{-1}). Considering the same previous depth interval, I4 area had similar values to NV areas (mean of 3.7 Mg ha^{-1}) (Figure 3). Higher soil C stock was found in the NV than I6 area, being statistical different in the depth interval from 50 to 100 cm (Figure 3b) while the other layers did not differ statistical significantly.

The increase soil C stock due to the replacement of NV by sugarcane in I4 area was 0.95 and $0.33 \text{ Mg ha}^{-1} \text{ y}^{-1}$ for 0-30 and 0-100 cm depths, respectively. On the other hand, the I6 area showed a decrease in soil C stock relative to the NV area for 0-100 cm depth, there was a decrease in soil C stocks of $0.71 \text{ Mg ha}^{-1} \text{ y}^{-1}$ for 1 me, while the 0-30 cm depth also had a slight increase of $0.33 \text{ Mg ha}^{-1} \text{ y}^{-1}$ relative to the NV area.

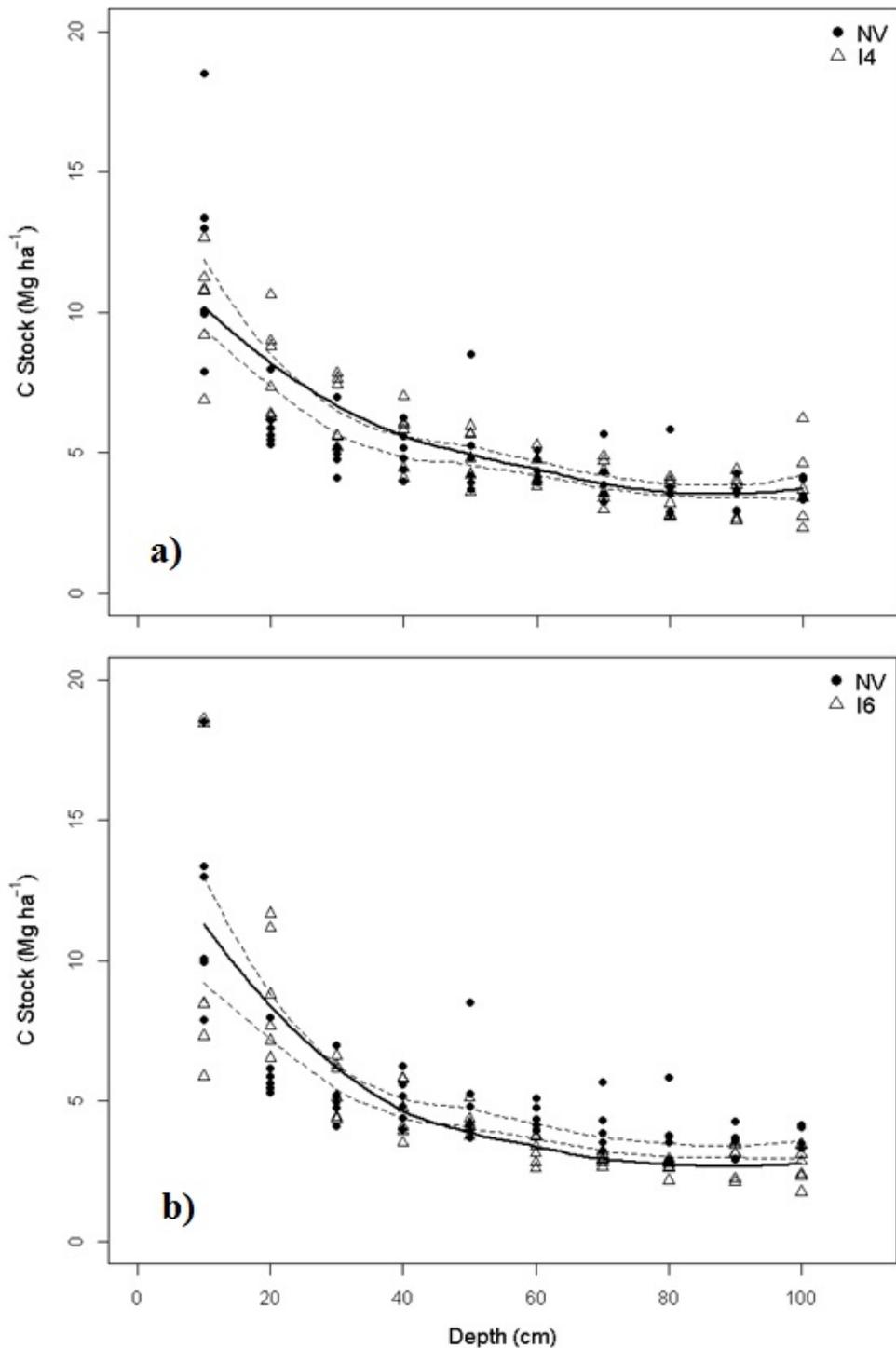


Figure 3 - Soil C stock (Mg ha⁻¹) between 0 – 100 cm depth in a) native vegetation (NV) (filled circles) and sugarcane I4 (empty triangles) land use and in b) native vegetation (NV) (filled circles) and sugarcane I6 (empty triangles). Dashed lines represent upper and lower bounds of 95% confidence intervals from bootstrapped (n = 1000) loess regressions of combined NV and I4 data (a) and NV and I6 data (b). In a) solid lines represents loess regression of soil C stocks in I4 only, if this line sits outside the confidence interval it can be inferred that NV and I4 are significantly different, and b) solid lines represents loess regression of soil C stocks in I6 only, if this line sits outside the confidence interval it can be inferred that NV and I6 are significantly different

3.3.2 Carbon in soil fractions

The physical fractionation proved to be an efficient tool in order to quantify the SOM fractions without significant losses of samples, once the mean mass recovery found in this study was 97% (Table S1).

Higher C content was observed in the OMF at 0-10 cm depth in the I6 area compared of the other areas (NV and I4), though there was also higher standard deviation for the same area. In addition, NV and I4 showed similar result for all layers and fractions measured. Even so, both sugarcane areas showed a slight increase in the C content for OMF in the 10-20 cm depth in compared to NV. Both sugarcane areas and NV had the low C contents in the SF. Carbon in the SCF and SF was similar in all land uses.

Despite the small increase in the OMF noted in the layer 0-10 and 10-20 cm under sugarcane fields, there were no statistical differences comparing irrigation sugarcane fields and NV, as well as no differences in the other depths and fractions assessed ($P>0.05$) (Figure 4).

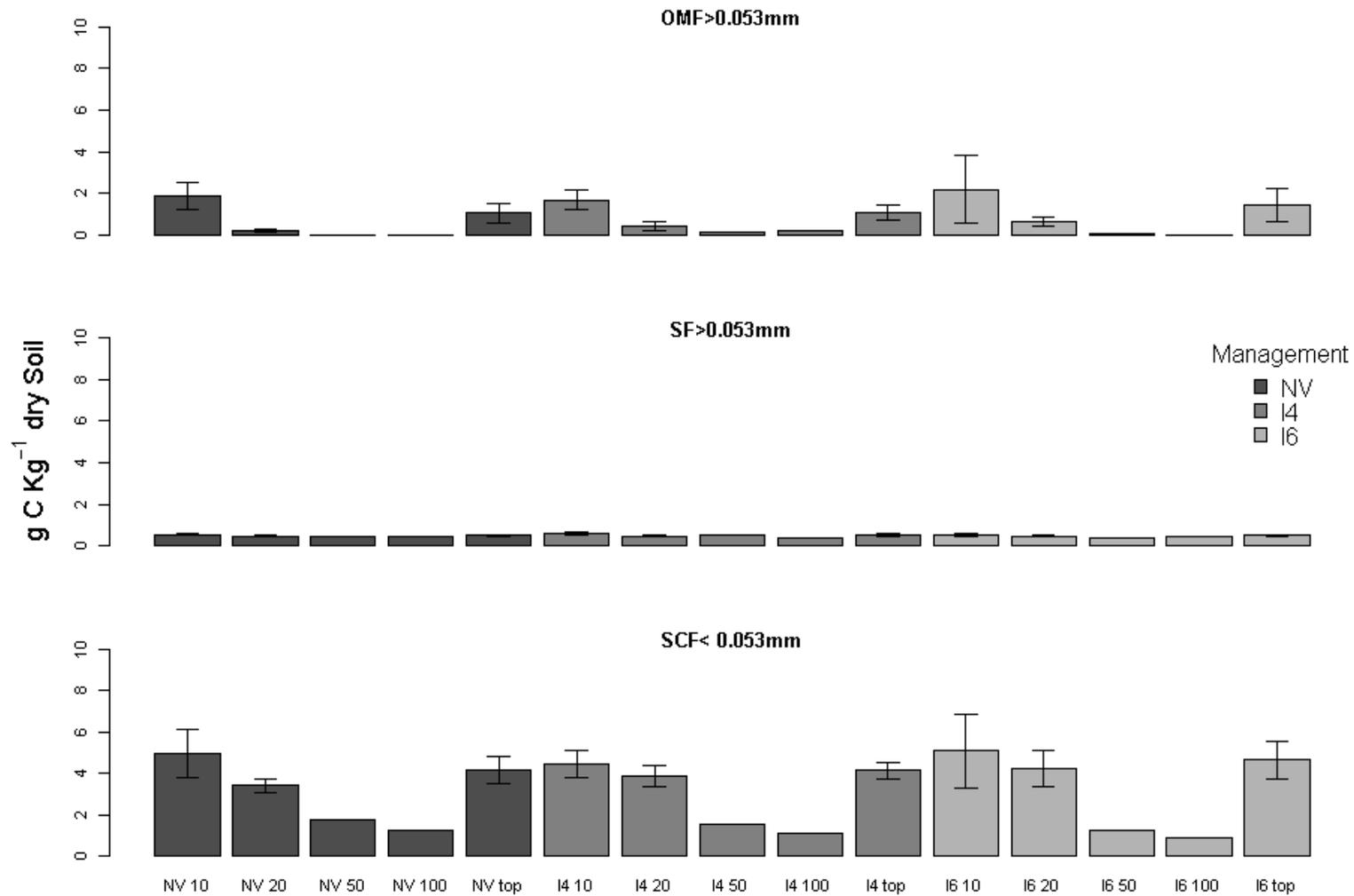


Figure 4 - Soil C content (g kg⁻¹ dry soil) in organic matter (OMF), sand (SF) and silt+clay (SCF) soil fractions. (NV) native vegetation, (I4) irrigation four years, (I6) irrigation six year. Top means 0-20 cm depth. Vertical bars show ±1 standard error (n = 3)

3.3.3 Modelling soil carbon and long-term scenarios

The modelling approach can give an enhanced understanding of the effects of irrigation in Brazilian sugarcane production. In addition it was possible to provide, with the aid of the Century model, the length of the time necessary to achieve a steady-state in soil C stocks under an irrigated sugarcane system.

The model showed a good fit between simulated vs measured values at 0-20 cm depth for all sites assessed (r value = 0.94 and RSME = 0.41; Figure 5). The maximum variation found between them was 0.59 Mg ha^{-1} , suggesting a good performance. As highlighted previously in the measured values, the simulated results also showed for both I4 or I6 areas, higher soil C stock (18.85 and 19.56 Mg ha^{-1} respectively) than NV area (16.33 Mg ha^{-1}).

Indeed the LUC led to decreases in soil C stock ($-1.2 \text{ Mg C ha}^{-1}$) in the first years for both sugarcane areas of the chronosequence. Nonetheless, after only 4 years under irrigation, it is possible to note that the soil C stock losses were replaced surpassing the NV levels, with mean increases of $0.3 \text{ Mg ha}^{-1} \text{ y}^{-1}$. After 6 years under irrigated sugarcane the gains was higher, at around $0.5 \text{ ha}^{-1} \text{ y}^{-1}$, thus greater than the measured results. Even so, taking into account only four years of the I6 area the gains were the same as those under the I4 area, $0.3 \text{ Mg ha}^{-1} \text{ y}^{-1}$. The differences between values measured and simulated are 0.37; 0.18 and 0.59 Mg ha^{-1} for NV, I4 and I6 simulated and measured, respectively (Figure 5).

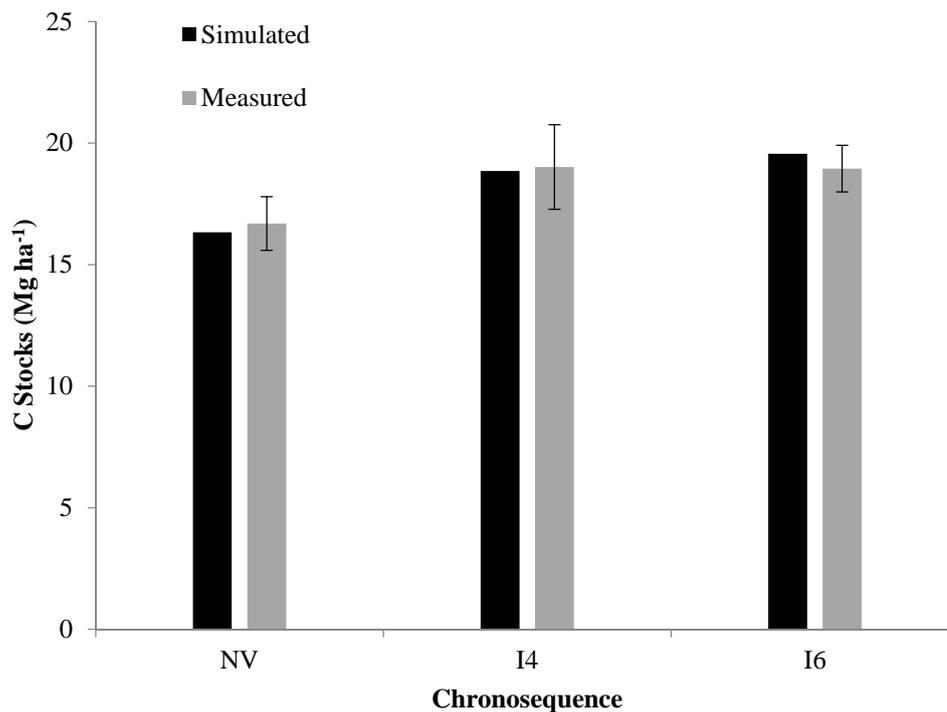


Figure 5 - Simulated and measured results for soil carbon stock (0-20 cm) for the chronosequence land uses assessed. (NV) native vegetation, (I4) irrigation four years, (I6) irrigation six year. Vertical bars show ± 1 standard error (n = 12)

In this way, the results of the modelling showed a good relationship between the chronosequence areas with similarity between the soil types. Simulated soil C stocks were similar to each other for the irrigated areas, as also previously observed between measured values. The small difference between them potentially occurred owing to the short time between ages of the sugarcane fields assessed. We assumed that the simulations were consistent with measured values, and therefore the long-term approaches were carried out up to the maximum soil C stock would be reached (achieved on year of 2055). Subsequently the simulation was continued to the year 2100, to demonstrate that the equilibrium state was achieved (Figure 6).

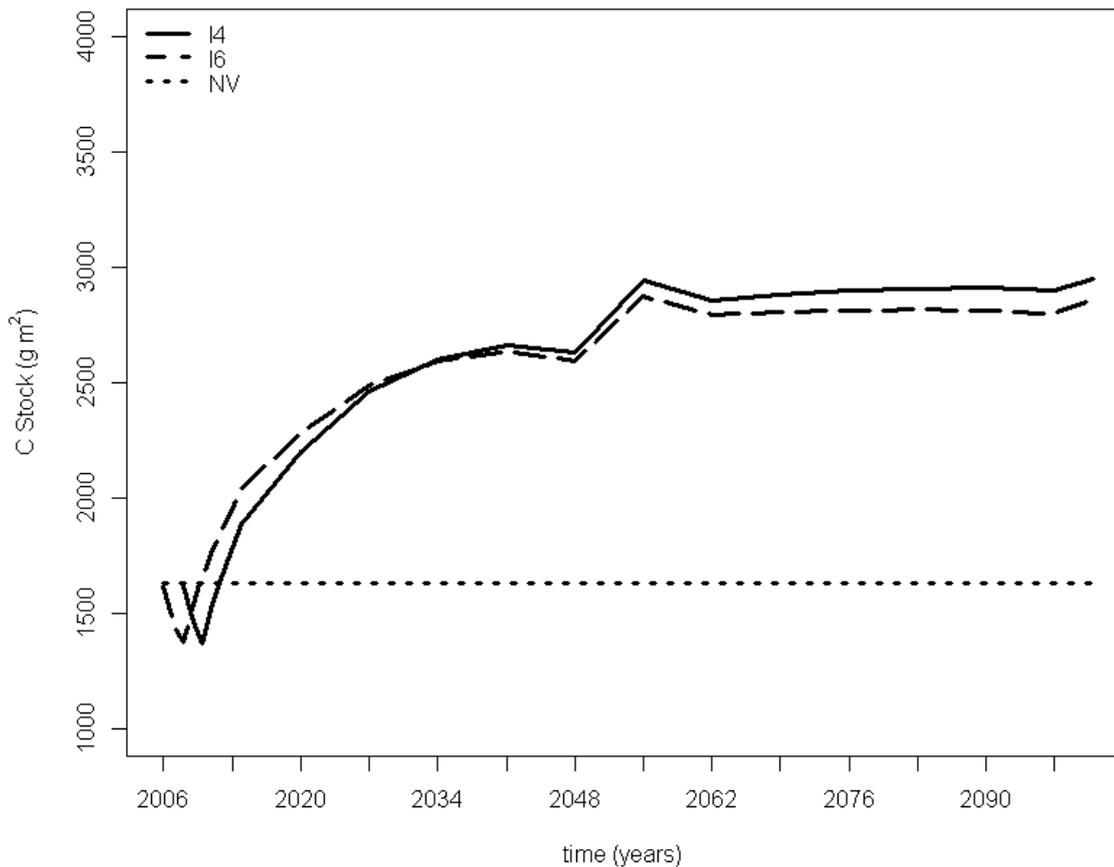


Figure 6 - Long term approaches over 100 years projection for land use changes NV (native vegetation) to sugarcane in each 10 years. (I4) irrigation four year, (I6) irrigation six year

Gains in soil C stocks up to the equilibrium state (2050) was around $0.22 \text{ Mg ha}^{-1} \text{ y}^{-1}$ in the I6 area and $0.28 \text{ Mg ha}^{-1} \text{ y}^{-1}$ for the I4 area whereas the rate of increase after this year was virtually zero, with values of around $0.001 \text{ Mg ha}^{-1} \text{ y}^{-1}$ for both sugarcane areas and variation of $-0.003 \text{ Mg ha}^{-1} \text{ y}^{-1}$.

The overall rate of increase considering the whole time evaluated (2100) was smaller than those found for the first years after replacement of native vegetation. Irrigation sugarcane resulted in gains of around $0.1 \text{ Mg ha}^{-1} \text{ y}^{-1}$. The total increase was 12 Mg ha^{-1} for the I6 area and 13 Mg ha^{-1} for the I4 area by 2100 relative to the NV. The differences between the sugarcane areas in the final of the simulations were also pronounced with a slight increase for I4 in relation to I6 up to around 2050, where the equilibrium state was reached for both sugarcane areas.

3.4. Discussion

Based on the forecast of GOLDEMBERG et al. (2014) it is expected that the area of sugarcane fields in Brazil will expand by 6.4 Mha by 2021. In Brazil it is presumed that over the next six years much of this expansion will be in semi-arid areas, so that it should occur especially on degraded pasture and not under native vegetation (MANZATTO et al., 2009). Assessing the impact on soil C stock of the expansion of sugarcane into these semi-arid areas is essential for development of crop in this region and to support the decisions of public policy makers. There is, however, lack of information in the literature about the impacts on soil C stock of sugarcane production in these semi-arid areas, which will require significant changes in management systems including the need for irrigation systems.

Low rainfall and therefore reduced soil water content will limit evapotranspiration which result in lower yields (CABRAL et al., 2012). Waclawovsky et al. (2010) found that irrigation resulted in higher yield of sugarcane, in an area with lower precipitation, lower cloudiness and higher solar radiation than most sugarcane areas of Brazil. Monteiro and Sentelhas (2014) suggest that based on model simulation, in areas where water stress is pronounced, an 83% increase in yield could be achieved through a full-irrigated system. Above and belowground yields are positively associated with soil C stock as increase in yields results in increased inputs into the soil, thus irrigation of sugarcane in dry areas would be expected to result in increases in soil C stocks compared to non-irrigated production (FOLLET, 2001). Few studies have, however, directly tested this hypothesis.

Soil C stocks were increased after the replacement of NV drylands with irrigated sugarcane, especially in the surface soil (0-30 cm). However the increase was only significant in one of the two areas, I4. Although, the older area (I6) has an increase of 2 Mg ha⁻¹ for the surface layers, it does not differ significantly to the NV dryland. Differences in soil C stocks may have been found in I4 but not in I6 due to two main factors: First, native vegetation from semi-arid is known to have a higher belowground biomass to aboveground ratio (BGB:AGB), than sugarcane, this could potential lead to higher C inputs especially in the early years post LUC, from root turn over and exudates. Second, irrigation of the sugarcane may have enhanced decomposition rates by boosting microbial activity and stimulating mineralisation, thereby limiting increases in soil C stock (SCHIPPER et al., 2012; GIUBERGIA et al., 2013). The fact that the I6 area had two years more irrigation than the I4 area,

could perhaps have been affected by both the presence of belowground biomass coming from native vegetation and higher microbial activity. According to Paul and Clark (1996) soil moisture can boost microbial activity and thus is an important factor controlling organic matter cycling. In agreement with this, increases in CO₂ fluxes have been reported in areas subject to irrigation practices (JABRO et al., 2008; SAINJU et al., 2008), which reinforces the suggested increase in microbial activities at these areas.

The modelling approach also provided some important insights into the factors affecting the soil C stock. The modelling suggested that, at least for the modelled surface soil (0-20cm), both sugarcane areas had the same rate of increase in soil C stocks in the first four years after LUC. In addition, the parameter in the Century outputs related to microbial biomass ((som1c(2))) also indicated similar increases in both sugarcane areas compared to the NV dryland, which was likely to have been driven by the irrigation practices. On the other hand, the measurement data suggest that after this initial four years the rate of changes in soil C stocks between the two sugarcane areas diverged, as increases in soil C stocks compared to the NV were less for I6 than for I4. Based on the modelled results we suggest that this divergence was caused because the irrigation levels in I6 area were after this initial 4 years sufficient to lead to a microbial mineralisation rate that was either greater or equivalent to soil C stock accumulation for surface layers (0-20 cm).

Following LUC change in soil C stock can take decadal scales to reach a new equilibrium (TORNQUIST et al. 2009; BORTOLON et al. 2011; BRANDANI et al. 2014). Thus it is important to consider changes past the initial 6 years, as shorter term study might either under or overestimate the soil C stock. Our long-term predictions, indicated that despite of the potential increased on microbial activity, soil C stocks will continue to increase in both irrigated sugarcane areas for at least 42 years (gains of 0.28 and 0.22 Mg ha⁻¹ y⁻¹, for I4 and I6 respectively). These annual accumulation rates are higher than those found by Lal et al. (1998) (0.05 to 0.15 Mg C ha⁻¹ y⁻¹) but are similar than those reported by Giubergia et al. (2013) of 0.22 Mg ha⁻¹ y⁻¹ for a long-term study on semi-arid region under an area that followed wheat, soybean and maize. However, the climate parameters used in this study for the long-term scenarios were a mean climate conditions, and management practices including irrigation were kept static. This may not match reality and, for example irrigation, practices may change over time. This could impact microbial activities,

and if the model is predicting higher of irrigation that is occurring this would possibly result in lower soil C stocks accumulation rates than predicted.

Wu et al. (2008) also studied a long-term irrigation practice under cultivated fields and stated that fast accumulation in soil C stocks occurred in at 10-25 cm as result of incorporation organic matter during tillage and through inputs from crop roots. Tillage in our long-term predictions, was simulated every 7 years, this was linked to a modelled increase in soil C stock. The modelled increases in soil C in the surface soil predicted by the model also fit with measured findings of other, which have suggested increases in soil C stock for the surface layers even in field conditions with high C decomposition rates (GILLABEL et al., 2007; DENEFT et al., 2008; GELAW et al., 2014). As was found in the modelling and field measurements of this study, Deneft et al. (2008) also found increased soil C stocks in the surface soil (0-20 cm) due to the use of pivot-irrigating in a cultivated system compared to a dryland cultivated area. They found an increase of 10-13% in soil C stocks, similar to the difference found here between both sugar cane areas compared to the NV (13%). Yet, the authors highlighted that soil inorganic carbon (SIC) achieved roughly half of the total C stocks found, when considering 0-75 cm depth, with differences over this depth being directly driven by changes in SIC (DENEFT et al., 2008). Most authors who assessed irrigation on arid or semi-arid regions also pointed out the importance of SIC stocks. Brazilian soils are characterized with low amounts of inorganic carbon, however based on these findings it would be important to assess SIC under irrigation in semi-arid region.

In contrast to our finding Condrón et al. (2014) in a long-term evaluation (62 years) at irrigated temperate grazed pasture, where soil disturbance is not usual practice, found significantly lower soil C stocks under irrigated system than dryland areas. The authors highlighted that irrigation led an increased primary production and inputs to the soil but also accelerated the decomposition of organic matter effectively putting a “brake” on C accumulation (CONDROÑ et al., 2014). The authors also suggest that in addition to changes in soil moisture, many other factors also influences the impacts on soil C stocks, including: plant residue quality, reduced root mass, high root turnover, higher soil invertebrate activity and a potential enhance leaching of soluble C. These factors are responsible for the either small increase or reduced in soil C stock in the surface and deeper soil layer. Taking in account these results, our modelling may have overestimated the rate of soil C accumulation,

as it does not take into account these potential influence of such factors indicated as a responsible for losses in soil C stocks.

Although potentially overestimated in the modelling approaches, the increase of soil C stocks on the surface layers is expected as the residue inputs and the root system are typically greatest in the top 20 cm of the soil profile (SILVA-OLAYA, 2014). Assessment of the deeper layers is also important as subsoil horizons may contribute more than half of the total soil C stocks (RUMPEL; KÖGEL-KNABNER, 2011). However, over the entire profile (0-100 cm) there were no difference in soil C stock between the sugarcane fields and the NV in spite a decrease of $-0.71 \text{ Mg ha}^{-1} \text{ y}^{-1}$ in I6 area compared to the NV.

Due to the different impacts on soil C stock with depth, we used a soil layers analysis (loess regression), which allows deeper layer to respond in an opposite direction to the surface layers. The soil layers approach was also used due to the potential variability on a cumulative assessment (KRAVCHENKO; ROBERTSON, 2011). Besides that, it is expected by analysing soil layers, older cultivation plus deeper layer may show an opposing effect to that found for upper layers. Results from this analysis highlighted that soil C stocks were higher in the 20-30 and 30-40 cm layers of the I4 than in the NV, which again suggests that the higher cumulative soil C stocks found in the sugarcane (I4) 0-30 cm might due to the incorporation of C from belowground biomass of the native vegetation after LUC. Differences in soil C stock were not found in the 0-10 and 10-20 cm further supporting this hypothesis. In addition irrigation system may have redistributed soil C stock in depth through leaching of soluble forms of C, which in turn could have also led to an increase below 20 cm depth as well as higher root biomass at this depth (KELLIHER et al., 2012; GIUBERGIA et al., 2013).

In contrast to the I4 area, but in agreement with our prior forecast, soil layers assessment showed the opposite results for I6 area, with less soil C stock in the deeper layers (50-100 cm) in relation to NV area while surface layers did not differ (Figure 3b). This result suggests that irrigation could decrease the soil C stock in long-term especially on deeper layers. In a study of temperate grazed pasture Condrón et al. (2014) also emphasize that there were decreases in soil C stock from 50 – 100 cm depth in the pasture irrigation treatment. This fits with findings for the sugarcane field in this study. In addition, typically on dry soils rooting depths must be deep to reach nutrients and available water, whereas on irrigated system roots tends

to concentrate in the top layers (SCHIPPER et al., 2012). We suggest that this may have had been an important factor in causing the lower soil C stock in the deep layers of the I6 area compared to the NV dryland. The potential losses in soil C stocks in the deeper layers could also occurred as result of higher leaching of soluble forms of C, particularly under sandy soils. This would fit with finding of Follet et al. (2013) who in their study of irrigated corn, even under clays soil, concluded that most soil C stock losses following conversion were from older C3 stocks. Further evaluations are required in order to access the impact of irrigation on movements as dissolved C on sugarcane fields. The lack of differences between surface layers of I6 area and NV dryland could be also due to the liming. I6 area was limed once whilst I4 was not anytime this may have reduced soil C stock decomposition in surface layers at I6 area due changes in microbial communities (RANGEL-CASTRO et al., 2005).

In addition, to changes in soil C stock irrigation may require other management practices which in turn may also affect soil C quality as well as stability (CHRISTENSEN, 2001; CONDRON et al. 2014). Condron et al. (2014) found that light fractions (OMF) decrease under irrigation in respect to dryland reference, and they attributed this to accelerated decomposition under irrigation. Opposite results were found here, with OMF in the sugarcane areas being somewhat higher than in the NV areas, although the differences were not significant. Potentially this occurred because of the higher annual dry matter inputs (especially by straw) in the sugarcane fields compared with the native vegetation (dryland). Although in OMF are often seasonal and spatial variable due to it the sensitivity of this fraction to organic residues inputs, this should be taking into account (CHRISTENSEN, 1992). The highest values of C were found in the SCF highlight that even though this fraction is small, the protection of aggregates by silt and clay contents is an important key to "trap" soil C. This is an important finding since this fraction has a long residence time and is relatively insensitive to impacts of land management (LISBOA et al., 2009).

In summary, though an increase in soil C stock was measured in the surface soil of the irrigated sugarcane compared to the NV, the increase was less prominent than we expected. This could have resulted from higher belowground C inputs from native vegetation in the early years after LUC, together with increased decomposition rates in the irrigated sugarcane fields. Based on our measurements of soil C stocks and the finding of others, we reject the hypothesis that increased yield will lead to an exponential increase soil C stock under irrigated sugarcane after direct LUC from dry

native vegetation. Yet, our modelling approaches indicated that there will be an increase for surface layers (0-20 cm) of up to $0.3 \text{ Mg ha}^{-1} \text{ y}^{-1}$ at least until the equilibrium state is reached which may take up to 40 years, although we recognize that increases in soil C stock under irrigation sugarcane will certainly be far from linear. Finally it is worth highlighting some main points: this is a new practice in Brazil, thus our measured results are from relatively "young" plantations make a straightforward conclusion difficult. Even so, it seems this is the first evaluation that assessed measured soil C stock data, quality of C linked of the MOS by fractionation techniques, and providing future scenarios of changes in soil C stocks under irrigated sugarcane fields. By means of these, we conclude that irrigation sugarcane system could induce soil C stock increase in the surface layers of soils but may also cause losses in deeper layers. Further research is needed especially on semi-arid region, to test our simulated long-term prediction on a range of soils and also for the whole soil profile and in order to improve our understanding of the mechanisms underlying these changes. We identify five main areas of work: i) Evaluating C4 inputs and potential C3 losses by isotopic techniques; ii) Evaluating impact on soil C stock plus tillage of sugarcane fields; iii) Evaluation of the relationship between increase yields and soil C stock following from others LU (pasture, croplands); iv) Perform an evaluation of soil C stock that considering SIC contents on the soil and v) Evaluating the potential GHG due to the use of this system.

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Supporting information

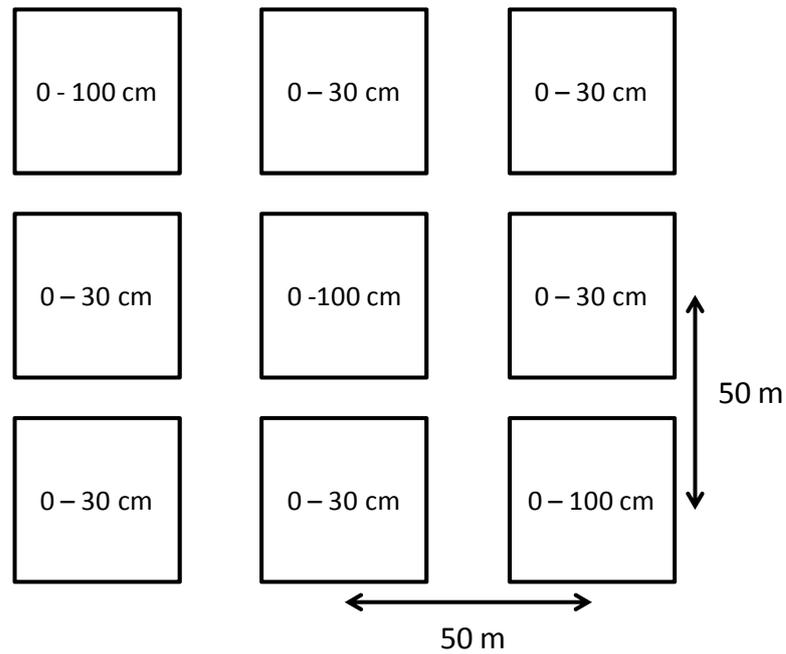


Figure S1. Experimental design of the soil sampling adapted from Mello et al. (2014).

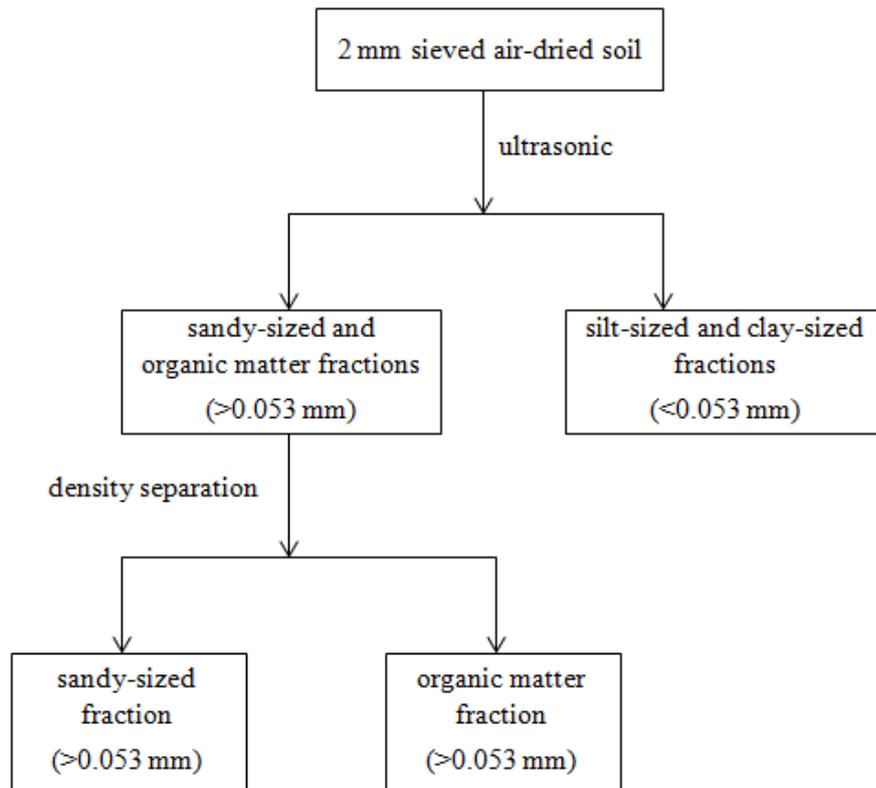


Figure S2. Scheme of the stages of physical fractionation

Table S1. Mass soil in each of SOM of the areas cultivated with irrigated sugarcane in Jaíba (MG) under different Land uses (NV: Native vegetation; I4: irrigation for four years; I6: irrigation for six year).

Reference area	Depth cm	OMF	SF	SCF	Mass recovery
		(>0.053 mm)	(>0.053 mm)	(<0.053 mm)	
----- g dry soil ⁻¹ -----					
NV	0-10	0.11	15.84	4.02	19.97
NV	0-10	0.30	17.20	2.24	19.74
NV	0-10	0.18	18.19	1.19	19.56
NV	10-20	0.02	17.56	2.36	19.94
NV	10-20	0.04	17.15	2.55	19.74
NV	10-20	0.02	17.53	2.43	19.98
NV	40-50	0.01	16.25	3.67	19.93
NV	90-100	0.01	15.16	4.40	19.57
I4	0-10	0.26	17.28	2.24	19.78
I4	0-10	0.12	17.32	2.22	19.66
I4	0-10	0.24	15.15	4.51	19.90
I4	10-20	0.19	16.82	2.24	19.25
I4	10-20	0.02	17.55	2.31	19.88
I4	10-20	0.12	15.58	4.18	19.88
I4	40-50	0.03	17.21	2.58	19.82
I4	90-100	0.12	15.99	3.54	19.65
I6	0-10	0.15	17.38	2.36	19.89
I6	0-10	0.57	16.86	2.30	19.73
I6	0-10	0.07	17.80	2.07	19.94
I6	10-20	0.06	17.22	2.58	19.86
I6	10-20	0.17	17.48	2.33	19.98
I6	10-20	0.06	17.18	2.65	19.89
I6	40-50	0.05	16.13	3.66	19.84
I6	90-100	0.01	16.03	3.94	19.98

4. FINAL CONSIDERATIONS

The main goal of this research was provided a better knowledge about soil C stock changes in response to the main management change on sugarcane production in Brazil. We also evaluated the potential impacts of the most promising management, i.e. the use of irrigation water in semi-arid region. This is the first data for Brazil accessing the sugarcane management change at same local/area and evaluating the effect of irrigation system. We performed evaluations of soil C stocks (1 metre depth), particle-size soil fractions (by physical soil C fractionation) and long-term approaches by model techniques.

The evaluation of soil C stocks changes suggested that the use of practices considered sustainable would lead to an increase in soil C stocks. Vinasse application was beneficial mainly for topsoil layer (0-30 cm depth), indicating an accumulation of $0.55 \text{ Mg C ha}^{-1} \text{ y}^{-1}$ for a period of 10 years whereas the implementation of unburned system showed an increase especially for the interval from 30 to 60 cm depth, with gains of $0.75 \text{ Mg C ha}^{-1} \text{ y}^{-1}$ for a period of 12 years. No differences were found for more than 30 cm depth of vinasse application and for the topsoil layers under UB. These results should have been related to the potential increase root biomass in surface layers and replanting operations for the first and second management, respectively. Particle-size fractions demonstrated to be a positive indicator for response in the management changes. Sites with recent soil disturbance (NV, V, UB) showed lower C linked of OMF although it also may be resulted from accelerated decomposition process at such fraction. On the other hand both management transition may contribute on the C accumulation linkage with SCF on topsoil layers (0-20 cm) which is responsible for more stable forms of the C. Modelling approaches showed an increase for both cases, however vinasse application was not the key driver on increase rates, while unburned system increased to almost 40% of the soil C stocks topsoil layers until equilibrium state was reached (after 36 years). These results showed that both transitions are a key factor to sustainable expansion of sugarcane production as well as to future environmental policies.

The use of irrigation system on semi-arid regions indicated a slight increase on soil C stock topsoil layers; however it was less prominent than expected probably due to the “young” conversion. Cumulative measured values of soil C stock and C

content at particle size fraction did not differ between native vegetation dry-land and irrigation sugarcane fields. Nevertheless seems that irrigation induce soil C stock increase for topsoil layers and could provide losses for deep layers. Simulated long-term evaluations showed an increase of $0.3 \text{ Mg ha}^{-1} \text{ y}^{-1}$ during at least 40 years for 0-20 cm depth. Therefore, future researches for test our long-term prediction on a range of soil and investigating the mechanisms these changes are required as well as aiming feasibility and economic resources.

Due to the growing concerns about global warming and climate changes, there is an emerging international policies and regulations in order to assess the sustainability of the supply chains. The evaluation in soil C stocks is an important part in the life-cycle analyses of agricultural products. The significant increase of soil C stock due to management change found in this study must be considered in future life-cycle analyses. Highlighting that evaluation in deep layers, which are omitted by the IPCC, must also be considered since it may have a higher potential to avoid soil C losses for atmosphere than topsoil layers. Besides that, this research indicated that management change for improvement practices may also reduce the payback time for sugarcane ethanol recently found by Mello et al. (2014) whereas may face the current debates about expansion and energy demand (GOLDEMBERG et al., 2014) potentially delivering low-carbon renewable fuels through such improved management. The results must be taken into consideration when it comes to the carbon footprint of the ethanol.

Finally, the results generated here can be used as a basis for other scientific studies especially for management change and irrigation sugarcane fields reporting the sustainability potential of sugarcane ethanol. The results can also be useful to drive the public decision makers, stakeholders and sugarcane private sector in the establishment of appropriate management strategies for growing and development of sugarcane chain.