Nutr Cycl Agroecosyst
DOI 10.1007/s10705-016-9812-z

ORIGINAL ARTICLE

Changes in soil organic carbon during 22 years of pastures, cropping or integrated crop/livestock systems in the Brazilian Cerrado

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Received: 25 May 2016 / Accepted: 18 November 2016

Abstract In Brazil's central savanna region, government policy is to encourage the conversion of conventional plough tillage (PT) agriculture to no-till (NT) and raise the productivity of under-utilized pastures, including their conversion to integrated crop-livestock (ICL) systems, with the objective of increasing soil organic carbon (SOC) at the expense of atmospheric carbon dioxide. An experiment was established in 1991 by liming and fertilizing at two levels an area of native vegetation (NV). The treatments, replicated in randomized plots, included pastures, continuous cropping and ICL systems under PT or NT. The aim of this study was to quantify the SOC accumulation to 100 cm depth under these treatments over time. The high C:N ratios suggested that there was a high proportion of charcoal present in the soil. Increasing fertilizer inputs had no overall significant effect on SOC stocks. Stocks of SOC changed little under pastures. Analyses of ^{13}C abundance showed that higher fertilizer inputs increased the decomposition rate of C derived from NV under pure grass pastures. Continuous cropping under NT preserved SOC and under PT there were significant losses. The highest SOC stocks were found under ILP treatments, but not all ILP treatments accumulated SOC even under NT. These results indicate that government initiatives to substitute PT with NT and to intensify beef cattle production will have only modest short-term gains in SOC accumulation.

Keywords ^{13}C abundance · Carbon sequestration · Cerrado region · Charcoal · Forage legume · Integrated crop livestock systems · Soil carbon · Tropical pastures

Electronic supplementary material The online version of this article (doi:10.1007/s10705-016-9812-z) contains supplementary material, which is available to authorized users.

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Introduction

The Brazilian government pledged at COP 15 in Copenhagen in 2010 as nationally appropriate mitigation actions (NAMAs), the adoption of integrated crop-livestock (ICL) systems (range of estimated reduction of greenhouse gases [GHG] equivalent to 18–22 Tg [millions of tonnes] of CO_2 [CO_2eq] until 2020) and recovery of degraded pastures (range of estimated reduction: 83–104 Tg of CO_2eq until 2020).

Subsequently at COP21 in Paris in 2015, the Brazilian government presented their Intended Nationally Determined Contributions (iNDCs) for the agriculture sector to strengthen the Low Carbon Agriculture (ABC) program as the main strategy for sustainable agriculture development. These iNDCs include the recovery of 15 million ha (Mha) of degraded pasturelands and the installation of 5 Mha of integrated crop-livestock-forestry systems (ICLF) by 2030. Expansion of cropping areas under no-tillage is also among the proposed NAMAs but not as an iNDC.

One of the pillars of the proposed reductions is the removal of CO_2 from the atmosphere by increasing stocks of soil carbon promoted by the recovery of degraded pastures or the introduction of integrated crop/livestock systems. Most studies conducted in several of Brazil's important cattle-producing regions strongly indicate that soil C is higher under productive pastures than under degraded pastures (e.g. Neill et al. 1997; Nepstad et al. 1994; Braz et al. 2013). In addition, the soil-fertility status influences pasture productivity and consequently the accumulation of C in soil (Carvalho et al. 2010). For soils of the Central savannah of Brazil (Cerrado region) it was demonstrated that N is the most limiting nutrient for pasture growth but the supply of P, K and S also brought about increased pasture biomass production (Oliveira et al. 2001). The use of forage legumes to supply biologically fixed N_2 to the pasture is a possibility to avoid economic constraints with N fertilization and has the potential to stock more C in soil than low yielding pastures (Tarré et al. 2001; Mortenson et al. 2004). However, the use of mixed grass/legume pastures in Brazil is not common and persistence of the legume in such swards is generally the principal problem to maintain the N input from the legume (Boddey et al. 2015).

ICL systems were proposed to recover pastures with the surplus nutrients derived from a preceding crop treated with fertilizers, the cost of which are abated by commercializing the grain produced. Results on the impact on soil C stocks of the conversion of degraded pastures to ICL systems are rare. Salton et al. (2014) examined the soil layer of 0–20 cm of a long-term experiment and verified that soil C stocks reached the highest levels in a pure well-managed pasture followed by soybean based ICL systems with crops managed under no-tillage. They also showed that the use of continuous cropping under plough tillage (PT) resulted in the lowest soil C stocks. Apart from adjusting C and N inputs, the use of no-tillage has the potential to enhance soil C stocks in cropping systems (Boddey et al. 2010), and this was also defended by Carvalho et al. (2010) when comparing ICL systems.

The studies on soil C stocks have been based on the comparison of neighbouring areas of productive and degraded pastures and often also the stocks of soil C under remaining native vegetation—the chronosequence approach. Using this approach in the Cerrado region, higher soil C stocks were reported under ungrazed or lightly grazed pastures than under the native vegetation (Corazza et al. 1999; Chapuis-Lardy et al. 2002; da Silva et al. 2004). More recently, Braz et al. (2013) published a chronosequence study performed in the Cerrado region comparing areas of native vegetation with adjacent productive and degraded pastures. They concluded that pastures that were indicated to be more productive by, *inter-alia*, their regrowth rate and rates of litter deposition, showed higher soil C stocks than those under the natural vegetation and also above those under degraded pastures. These differences were greater in soils of higher clay content.

The chronosequence approach requires that the land use under comparison are located in areas presenting the same soil type and management history. As this is not easy to prove, the use of long-term experiments where soil samples were taken at the time of establishment and analysed and stored are regarded the "gold standard" to assess changes in soil C (and N) stocks over time (Smith et al. 2012). Such studies do not appear to have been performed in Brazil, but medium term (10–20 years) experiments with treatments comparing different crop rotations under either PT and NT arranged in randomised block designs have shown that NT does preserve soil C compared to PT (e.g. Bayer et al. 2000, 2002; Sisti et al. 2004; Diekow et al. 2005; Boddey et al. 2010). A similar conclusion was arrived at by Zotarelli et al. (2012), however, when the final soil C stocks of each crop rotation were compared with the stocks 12 years earlier in the same plot the conclusion was that the higher soil C stocks under NT were due to lower losses than those observed under PT and not gains under NT.

The objective of this study was to investigate the impact of different land-use systems: continuous pastures, continuous cropping or integrated crop/

livestock systems under two levels of soil fertility status on changes of soil C and N stocks 22 years after conversion of the land use from native Cerrado vegetation.

Materials and methods

The experiment was situated at the field station of the Embrapa Cerrados Centre (15°39′S, 47°44′W) near Planaltina (Federal District) which prior to installation was native Cerrado vegetation of the type Cerrado "sensu stricto" (Eiten 1972) consisting of shrubs and small trees in a continuous carpet of grasses. The soil in this area is classified by the US Soil Taxonomy system as an Oxisol (Typic Acrustox) or "Latossolo Vermelho" by the Brazilian classification. A fuller description of the soil at this site was given by Chapuis-Lardy et al. (2002) and the analysis of the soil under the native vegetation (NV) is given in Table 1.

In 1991 the area was cleared of NV and lime was added to the whole experimental area. The experiment consisted of eight basic land-use treatments at two fertilizer/lime levels (F1 and F2) arranged in a randomized complete block factorial design (8 land uses × 2 fertilizer levels). Two areas at each end of experiment remained as undisturbed native vegetation with no lime addition, giving four replicate areas of NV. For the lower fertility (F1) lime was added at 3.4 Mg ha^{-1} and for F2 at 5.8 Mg ha^{-1}. The land-use treatments were pastures, either pure grass or mixed with a forage legume, continuous cropping under no-till or plough tillage (PT—disc plough followed by a harrow), or integrated crop/livestock (ICL) system. There were eight land use treatments at each of the two fertility levels, which resulted in a factorial design 8 × 2, with four replicates. Land use treatments were as follows:

(a) Continuous pasture (grass monoculture)
(b) Continuous grass/legume mixed pasture
(c) Continuous cropping under NT
(d) Continuous cropping under PT
(e) ILC—4 years pasture followed by 4 years cropping with all crops and pastures implanted with NT.
(f) ILC—4 years pasture followed by 4 years cropping with all crops and pastures implanted with PT.
(g) ILC—4 years cropping followed by 4 years pasture with all crops and pastures implanted with NT.
(h) ILC—4 years cropping followed by 4 years pasture with all crops and pastures implanted with PT.

The sequence of crops/pasture species in the eight land-use systems over the 22-year period is given in Table 2. The total of all applications of fertilizers to each treatment is given in the Table 3. Each treatment had four replicate plots of 40 × 50 m (N = 4). In the continuous cropping treatments under NT and PT, just one crop per year was planted at the start of the rainy season (November). After harvest it was left fallow until the next year. In the PT treatment the residues of the crop after harvest were buried with a disc plough. Over the 22 years of cropping there were 14, 5, 2, and 1 years respectively, of soybean, maize, millet and sorghum (Table 2) always followed by fallow from the end of one rainy season until the start of the next.

In the ICL and the continuous pasture treatments, all pastures were grazed by Nellore cattle (*Bos indicus*), the total animal weight being regulated by introducing or removing animals such that the green forage on offer was between approximately 8–10 kg per 100 kg of animal live weight.

Soil sampling

Three sets of soil samples were taken from the pasture, ICL and continuously cropped plots in and four areas of the neighbouring native vegetation (NV) in 2013. In 2001 one set of four undeformed samples were taken from each plot to assess soil bulk density by using a bevelled ring of known volume (84.9 cm^3) driven into each of the four the sides of sampling trenches dug to over 100 cm depth in 2001, as described by Sisti et al. (2004). The depth intervals were 0–5, 5–10, 10–20, 20–30, 30–40, 40–60, 60–80 and 80–100 cm. The samples were dried at 110 °C for the assessment of dry weight and subsequently soil bulk density.

In the years 2001, 2009 and 2013 samples were taken for analysis of total C and N content using a Dutch auger from the same depth intervals as for the evaluation of soil density at five points in each plot and in the four areas of NV and bulked to constitute one sample per depth interval per plot. In 2001 all plots of pasture treatments were sampled, but only plots of the

Table 1 Fertility analyses for the soil under the Cerrado native vegetation, the pastures and the continuous cropping taken in 2013

System	Depth	pH (H_2O)	Al cmolc/dm^3	Ca	Mg	H + Al	K mg/L	P
Cerrado native vegetation	0–10	4.8	0.7	0.5	0.3	9.1	58	1.7
	10–20	4.9	0.5	0.0	0.1	7.2	31	1.2
Pure grass pasture F1	0–10	5.5	0.1	1.7	0.7	6.6	99	1.8
	10–20	5.5	0.1	1.1	0.5	6.4	40	1.1
Pure grass pasture only F2	0–10	5.7	0.0	2.1	1.0	5.6	68	3.6
	10–20	5.6	0.0	1.6	0.7	4.8	21	0.8
Mixed grass/legume pasture F1	0–10	5.3	0.2	1.4	0.5	6.5	58	2.1
	10–20	5.3	0.1	1.1	0.4	5.9	26	1.5
Mixed grass/legume pasture F2	0–10	5.6	0.1	2.1	1.0	5.6	95	1.4
	10–20	5.5	0.1	1.6	0.7	5.6	51	0.9
Continuous cropping CT F1	0–10	4.7	0.5	0.7	0.1	8.3	80	4.1
	10–20	4.7	0.4	0.5	0.1	7.9	22	1.7
Continuous cropping CT F2	0–10	5.1	0.2	1.8	0.3	7.0	110	8.3
	10–20	5.1	0.2	1.3	0.3	6.3	42	2.4
Continuous cropping NT F1	0–10	5.2	0.2	1.7	0.5	7.2	57	4.5
	10–20	4.5	0.5	0.2	0.1	7.5	19	2.7
Continuous cropping NT F2	0–10	5.8	0.0	3.0	0.6	5.4	87	11.7
	10–20	4.8	0.3	0.7	0.2	7.4	27	3.1

Determinations made according to Embrapa (1997) Manual of soil analysis. Al and H + Al Titration, Ca and Mg by Atomic adsorption spectroscopy, K by flame photometry and P (Mehlich I) by colorimetry

higher fertility level (F2) were sampled for the ICL and continuous cropping. In 2009, plots of all F2 treatments were sampled. In 2013, all plots of both F1 and F2 and the NV were sampled at 5 points with an auger to all depth intervals to 100 cm. The bulked samples for each depth interval for each plot were air dried and then passed through a 2 mm sieve. Aliquots of the samples from the 0–30 cm depth intervals were taken for soil fertility analyses, and other aliquots were finely ground (<100 mesh) using a roller grinder similar to that described by Arnold and Schepers (2004).

Soil analyses

All bulked soil samples taken with the auger to 30 cm depth were analysed for pH, exchangeable Ca, Mg, Al and K and for available P (Mehlich I) using standard techniques (Embrapa 1997). Results of fertility analyses for the soil to depth of 20 cm under the NV, the pastures and the continuous cropping treatments are displayed in Table 1. Analyses of the fertility parameters under all treatments to 30 cm are in the supplementary information Table SI 01.

The concentrations of total N and C and the ^{13}C-isotopic abundance of the finely-ground soil samples taken in 2013 were determined on sub-samples containing between 200 and 400 µg total C using a continuous-flow isotope-ratio mass spectrometer (Finnigan DeltaPlus or Delta V mass spectrometer coupled to the output of a Costech (model ECS4010) total C and N analyser—Finnigan MAT, Bremen, Germany) in the “John Day Stable Isotope Laboratory” at Embrapa Agrobiologia (RJ). For the samples taken in 2001, the total C and N analyser used a Carlo Erba EA 1108, and for the samples taken in 2009 and 2013 using the Costech CN analyser. All samples taken to a depth of 30 cm in 2001 and 2009 were reanalysed simultaneously (randomised in the same carousels) with the 2013 samples using the Costech CN analyser. The N concentration in the samples was estimated for the 2001 and 2009 samples using the semi-micro Kjeldahl technique as described by Urquiaga et al. (1992).

Table 2 Sequence of crops in all treatments of the experiment from 1991 to 2014

Systems	Tillage	Fertility Regime	Sequence of crops[a] 1991	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002
Pure grass pasture	CT	F1 & F2	Ag	Ag	Ag	Ag	Ag	Ag	Ag	Ag	Ag	Bd	Bd	Bd
Mixed grass + legume pasture	CT	F1 & F2	Ag + Sg[b]	Ag + Sg	Ag + Sg	Ag + Sg	Ag + Sg	Ag + Sg	Ag + Sg	Ag + Sg	Ag + Sg	Bd + Sg	Bd + Sg	Bd + Sg
Rotation crop/pasture/crop (ICP)	CT	F1 & F2	So	So	Ma	So	Ag + Sg	Ag + Sg	Ag + Sg	Ag + Sg	So	Mt	So	Mt
	NT	F1 & F2	So	So	Ma	So	Ag + Sg	Ag + Sg	Ag + Sg	Ag + Sg	So	Mt	So	Mt
Rotation pasture/crop/pasture (IPC)	CT	F1 & F2	Ag + Sg	Ag + Sg	Ag + Sg	Ag + Sg	Ma	So	Ma	So	Pm	Pm	Pm	Pm
	NT	F1 & F2	Ag + Sg	Ag + Sg	Ag + Sg	Ag + Sg	Ma	So	Ma	So	Pm	Pm	Pm	Pm
Continuous cropping	CT	F1 & F2	So	So	Ma	So	Ma	So	Ma	So	So	Mt	So	Mt
	NT	F1 & F2	So	So	Ma	So	Ma	So	Ma	So	So	Mt	So	Mt
Cerrado natural vegetation														

Systems	Tillage	Fertility Regime	Sequence of crops[a] 2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014
Pure grass pasture	CT	F1 & F2	Bd	Bd	Bd	Bd	Bd	Bd	Bd	Bd	Bbp	Bbp	Bbp	Bbp
Mixed grass + legume pasture	CT	F1 & F2	Bd + Sg	Bd + Sg	Bd + Sg	Bd + Sg	Bd + Pp	Bd + Pp	Bd + Pp	Bd + Pp	Bbp	Bbp	Bbp	Bbp + Pp[b]
Rotation crop/pasture/crop (ICP)	CT	F1 & F2	So	So	Bb	Bb	So	Ma	So	Ma + Bbp	Bbp	Bbp	Bbp	So/Ma + Bbg
	NT	F1 & F2	So	So/Bb	Bb	Bb	So	Ma	So	Ma + Bbp	Bbp	Bbp	Bbp	So/Ma + Bbg
Rotation pasture/crop/pasture (IPC)	CT	F1 & F2	Pm	So	Sr	So	Bbp	Bbp	Bbp	Bbp	So	Ma	So/Sr + Bbp[b]	Bbp
	NT	F1 & F2	Pm	So	Sr	So	Bbp	Bbp	Bbp	Bbp	So	Ma	So/Sr + Bbp	Bbp
Continuous cropping	CT	F1 & F2	So	So	Sr	So	So	Ma	So	Ma	So	Ma	So/Sr + Bbp	So/Ma + Bbg
	NT	F1 & F2	So	So	Sr	So	So	Ma	So	Ma + Bbp	So	Ma + Bbp	So/Sr + Bbp	So/Ma + Bbg
Cerrado natural vegetation														

Ma Maize, *Mt* Millet cv CPAC 1, *So* soybean, *Sr* Sorghum, *Ag Andropogon gayanus* cv. Planaltina, *Bd Brachiaria decumbens* cv. Basilisk, *Bb Brachiaria brizantha* cv. Marandu, *Bbp Brachiaria brizantha* cv. Piatã, *Sg Stylosanthes guianensis* cv. Mineirão, *Pp* Pigeon pea (*Cajanus cajan*) cv. Mandarim, *Bbg Brachiaria brizantha* cv. Paiaguás, *Pm Panicum maximum* (Guinea grass)

[a] The year given indicates the year the crop was planted, the harvest was in the following year

[b] The symbol " + " indicates a mixture of species, and the symbol "/" crops in succession

Table 3 Total additions of lime and fertilizers from the start of the experiment (1991) until 2013

System	Lime ($Mg\ ha^{-1}$)	Gypsum	N[a] ($kg\ ha^{-1}$)	P[a]	K[a]	Micro[b]
Continous cropping						
F1	7.1	1.5	263	434	638	43
F2	10.6	4.3	443	842	1379	85
ICP (cropping start)						
F1	7.1	1.5	175	273	372	32
F2	10.6	4.3	240	527	816	63
IPC (pasture start)						
F1	7.1	1.5	243	252	357	41
F2	10.6	4.3	358	442	701	52
Continuous pastures						
F1	7.4	1.5	84[c]	93	120	30
F2	9.9	1.5	195[c]	111	169	30

[a] N added as urea, P as super-single phosphate and K as potassium chloride. Units as kg of nutrient element ha^{-1}

[b] Micro-nutrients Type BR = 12. Composed of 9% Zn, 1.8% B, 0.8% Cu, 2% Mn, 3.5% Fe, 0.1% Mo

[c] No N fertilizer was added to the mixed grass/legume pastures

Only samples from plots/paddocks of continuous pastures and the native Cerrado taken in 2001 and 2013 were analysed for ^{13}C abundance as the cropping sequences consisted of both C_3 and C_4 crops, which would make meaningful interpretation of ^{13}C abundance data virtually impossible.

Crop and pasture productivity

Crop productivity was estimated by harvesting five areas of 3 m^2 in each plot and the yields expressed in kg ha^{-1}. Animal live weight (LW) was monitored on all pastures throughout the experimental period in order to maintain the correct proportion (8–10%) of green material on offer with animal weight. However, as the number and race of cattle per paddock were frequently changed it was not often possible to allocate the live weight gain to a particular treatment or to calculate the LW gain per ha.

Calculations

Stocks of total N and C in the soil under each plot to 30 and 100 cm depth were calculated using the bulk density data for each plot expressed in Mg C or N ha^{-1}. As tillage operations and treading by grazing cattle compact the soil, the mass of soil to a 100 cm depth increases compared to the soil under the NV. To correct for this effect the procedure described by Ellert and Bettany (1995) was utilized applying the equation developed by Sisti et al. (2004).

It was assumed that soil compaction due to tillage and treading by cattle was most significant in the surface layers of the profiles so that the C and N stocks were calculated by subtracting the total C and N content of the extra weight of soil in the deepest (80–100 cm) layer sampled of each profile. This correction can be expressed mathematically as (Sisti et al. 2004):

$$Cs = \sum_{i=1}^{n-1} CTi + \left[MTn - \left(\sum_{i=1}^{n} MTi - \sum_{i=1}^{n} MSi \right) \right] CTn \quad (1)$$

where Cs is the total C stock (Mg C ha^{-1}) in soil to a depth equivalent to the same mass of soil as that in the reference profile (the NV area), $\sum_{i=1}^{n-1} CTi$ is the sum of the total carbon content (Mg ha^{-1}) in the layers 1 (surface) to layer 'n − 1' (penultimate 60–80 cm) in the treatment profile, $\sum_{i=1}^{n} MSi$ is the sum of the mass of soil (Mg ha^{-1}) in layer 1 (surface) to 'n' (100 cm) in the reference soil profile, $\sum_{i=1}^{n} MTi$ is the sum of the mass of soil (Mg ha^{-1}) in layer 1 (surface) to 'n' (greatest depth) in the treatment profile, M_{Tn} is the mass of soil in the deepest layer (80–100 cm) in the treatment profile and C_{Tn} is concentration of carbon

(Mg C Mg soil^{-1}) in the deepest layer in the treatment profile.

Statistical analyses

Analyses of variance (ANOVA) and the Student LSD test were used to separate land use treatments and soil fertility effects on soil C and N stocks of the most recent soil sampling (year 2013) using the software SISVAR, produced by the Federal University of Lavras (UFL), Lavras, Minas Gerais. In addition, soil C and N stocks for the years 1991, 2001, 2009 and 2013 within the same land use and soil fertility status were compared and separated by using the same tests but in this case considering the time as a single factor. The soil C and N stocks under native vegetation corresponded to the year 1991. Soil bulk densities measured in 2013 were compared among treatments within each soil layer also using ANOVA and the Student LSD test. In general, irrespective of existing a comparison, means of the measured variables were presented together with their standard errors.

Results and discussion

Crop yields

Data for crop yields from individual plots were not available for statistical comparisons to be made between treatments and systems in different years or over the whole 22-year period. Mean soybean yields under the continuous cropping produced between 2.26 and 2.37 Mg grain ha^{-1} under the low (F1) and higher (F2) fertilizer regimes. In the ILC systems these yields were respectively, 3.19 and 3.23 Mg ha^{-1} for F1 and F2 in the ICL that started with cropping, but lower at 2.10 and 2.12 Mg ha^{-1} in the ILC system that started with pasture. The difference here was mainly due to better rainfall in the years that soybean was planted in the ICL system. Soybean yields in commercial crop production in Brazil have increased over the last 22 years and for the Federal District where the experiment was carried out, ranged from <1.5 Mg ha^{-1} to approximately 3 Mg ha^{-1} today, so the yields registered in this study are within the range of local soybean producers. Maize was planted in only five of the 22 years of the study and yields varied widely from 4.2 to 8.6 Mg ha^{-1}. In some years

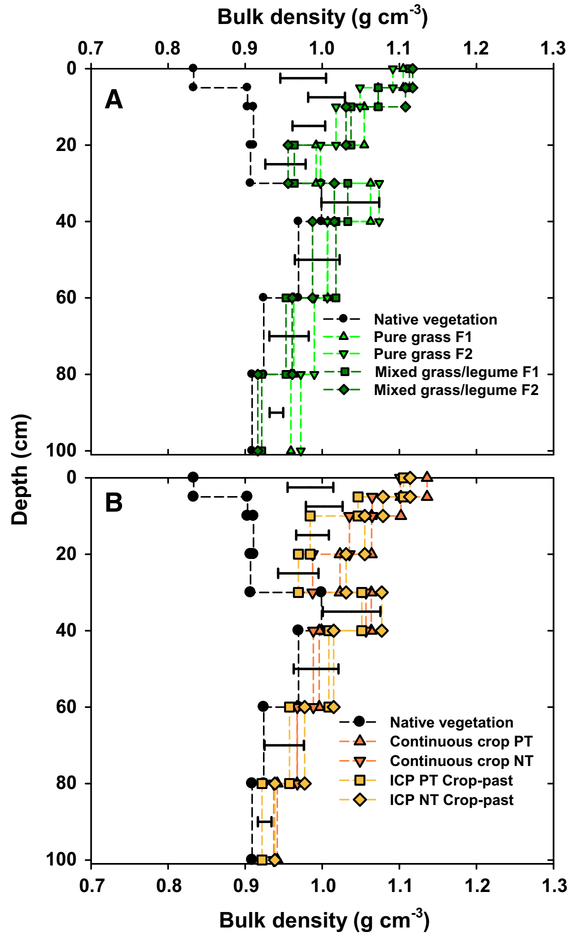


Fig. 1 Soil bulk density under the native Cerrado vegetation compared to the eight principal treatments in the experiment taken from the plots under the higher fertility treatment (F2) to a depth of 100 cm in eight depth intervals in 2001. *Error bars* represent the least significant difference at $P < 0.05$ (Student LSD test) between means of four replicates

owing to shortage of funding, fertilizer inputs were lower than planned.

Soil bulk density

The results clearly show that that mechanical operations such as ploughing and planting, and the installation and grazing of pastures, compacted the more superficial layers of the soil as witnessed by the increase in soil density (Fig. 1). Where farm machinery had been used (tillage or no-till planters) or pastures had been introduced the soil density was significantly increased ($P < 0.05$) to a depth of 30 cm

Table 4 Soil carbon concentration (g C kg soil^{-1}) in bulked samples from each depth interval to 100 cm from the Planaltina experiment in 2001, 2009 and 2013

Soil layer (cm)	NV	Pasture		Mixed pasture		ICL PT		ICL NT		ILC PT		ILC NT		Crop PT		Crop NT		LSD[a]
	C concentration (g kg^{-1})	Low	High	Low	High	Low	High	Low	High	Low	High	Low	High	Low	High	Low	High	
2001																		
0–5	29.7	31.3	24.1	30.4	29.9		26.1		28.1						25.2		25.5	4.9
5–10	26.6	26.7	22.6	27.2	26.5		25.2		24.9						24.1		22.4	3.6
10–20	20.4	22.1	20.3	23.4	23.5		21.4		22.0						22.3		21.8	2.9
20–30	17.7	17.8	14.4	16.8	19.6		17.6		18.7						15.9		20.4	2.1
30–40	15.1	14.9	13.1	13.2	15.0		14.7		15.3						13.1		16.0	2.3
40–60	12.7	12.3	10.5	12.4	12.5		11.5		11.7						10.8		11.1	1.6
60–80	10.7	10.3	9.2	10.6	10.5		10.1		10.5						9.4		9.5	1.5
80–100	9.1	8.8	8.5	8.8	9.1		8.6		9.0						8.0		8.3	1.2
2009																		
0–5	29.0		30.5		34.8		25.9		36.9		28.6		29.2		24.1		36.9	4.6
5–10	25.2		24.3		26.5		23.7		25.5		27.4		21.9		24.5		25.5	3.2
10–20	21.4		21.3		23.4		21.4		21.9		25.1		20.9		22.1		21.1	2.9
20–30	16.5		17.1		19.3		16.2		20.5		20.8		18.5		17.5		21.2	3.7
30–40	13.9		14.4		16.4		14.2		17.2		17.8		16.4		14.8		18.0	2.2
40–60	12.1		11.0		12.1		10.8		12.9		11.8		11.3		11.9		12.9	1.6
60–80	10.0		9.6		9.3		9.6		11.1		10.3		8.5		10.1		11.1	1.5
80–100	8.6		8.1		7.8		9.1		9.3		8.5		7.5		8.6		9.3	0.9
2013																		
0–5	37.6	29.0	28.0	27.5	30.2	26.2	26.6	35.4	37.8	30.3	29.1	28.4	29.5	23.9	23.3	28.2	31.1	5.2
5–10	30.0	27.5	23.9	26.7	29.5	23.9	24.2	25.6	26.2	27.5	28.0	23.3	22.8	22.5	24.0	25.4	22.6	5.0
10–20	21.4	21.5	21.6	20.0	23.0	22.9	20.0	21.4	22.8	22.9	25.4	19.5	21.2	19.4	21.3	20.5	21.0	3.6
20–30	18.0	18.5	14.6	17.2	18.9	17.1	16.4	17.5	19.7	19.4	20.6	19.1	19.2	16.4	17.8	18.2	18.2	3.7
30–40	15.7	14.3	12.7	15.2	15.9	15.0	13.8	14.8	16.4	16.1	16.7	15.1	15.2	14.1	14.2	15.5	16.4	2.7
40–60	12.9	12.3	11.4	12.4	13.1	12.6	11.6	11.9	13.4	13.1	13.3	12.2	11.7	11.5	11.9	13.3	12.2	2.2
60–80	11.1	10.6	9.0	10.0	9.9	10.0	9.8	9.8	11.1	10.8	11.1	9.6	9.6	9.4	10.0	10.3	10.3	1.6
80–100	9.4	9.4	7.2	8.7	9.0	9.2	8.7	8.6	9.1	9.4	9.9	8.5	8.8	8.5	8.5	8.6	8.8	1.5

Samples were taken from the Cerrado native vegetation (NV) on all three sampling dates, and in 2013 from all plots. In 2009 samples were taken from all high fertility plots, and in 2001 from all pastures and high fertility plots of ICL and continuous cropping

[a] LSD values represent the least significant differences between C concentrations (means of four replicates) in the same depth interval

Table 5 Soil nitrogen concentration (g N kg soil^{-1}) in bulked samples from each depth interval to 100 cm from the Planaltina experiment in 2001, 2009 and 2013

Soil layer (cm)	NV	Pasture		Mixed pasture		ICL PT		ICL NT		ILC PT		ILC NT		Crop PT		Crop NT		LSD[a]
	Soil N content (g kg^{-1})	Low	High	Low	High	Low	High	Low	High	Low	High	Low	High	Low	High	Low	High	
2001																		
0–5	1.61	1.70	1.33	1.64	1.72		1.38		1.68						1.37		1.39	0.29
5–10	1.39	1.43	1.25	1.50	1.52		1.34		1.42						1.27		1.20	0.21
10–20	1.05	1.14	1.05	1.28	1.30		1.15		1.11						1.18		1.19	0.13
20–30	0.92	0.91	0.84	0.93	1.12		0.96		1.01						0.84		1.07	0.10
30–40	0.79	0.76	0.73	0.73	0.81		0.80		0.82						0.71		0.83	0.12
40–60	0.67	0.65	0.63	0.64	0.66		0.62		0.63						0.57		0.58	0.08
60–80	0.57	0.56	0.51	0.54	0.56		0.54		0.57						0.50		0.51	0.06
80–100	0.46	0.46	0.46	0.45	0.46		0.46		0.46						0.43		0.42	0.04
2009																		
0–5	1.68		1.36		2.08		1.66		2.27		1.77		1.81		1.48		1.97	0.25
5–10	1.42		1.44		1.63		1.60		1.53		1.66		1.36		1.30		1.37	0.14
10–20	1.20		1.25		1.36		1.28		1.29		1.41		1.27		1.14		1.20	0.12
20–30	0.99		0.95		1.09		0.91		1.13		1.08		1.23		0.92		1.10	0.14
30–40	0.86		0.84		0.88		0.76		0.93		0.89		0.95		0.73		0.88	0.09
40–60	0.69		0.55		0.61		0.53		0.61		0.58		0.52		0.58		0.62	0.09
60–80	0.55		0.47		0.46		0.45		0.52		0.52		0.40		0.46		0.52	0.07
80–100	0.45		0.37		0.37		0.42		0.45		0.43		0.34		0.42		0.42	0.04
2013																		
0–5	2.25	1.68	1.66	1.65	1.75	1.72	1.81	2.49	2.71	1.75	1.77	1.85	2.07	1.48	1.50	2.02	2.25	0.41
5–10	1.75	1.58	1.43	1.58	1.66	1.48	1.45	1.53	1.58	1.57	1.90	1.33	1.42	1.31	1.38	1.54	1.36	0.30
10–20	1.17	1.28	1.24	1.29	1.24	1.47	1.18	1.24	1.35	1.28	1.46	1.14	1.26	0.98	1.14	1.13	1.20	0.28
20–30	0.95	1.06	0.92	1.00	1.04	0.96	0.91	0.98	1.15	1.03	1.15	1.04	1.09	0.82	0.93	0.99	1.00	0.20
30–40	0.76	0.82	0.72	0.85	0.82	0.82	0.75	0.86	0.98	0.83	0.89	0.78	0.85	0.71	0.74	0.78	0.94	0.18
40–60	0.59	0.59	0.60	0.61	0.62	0.62	0.62	0.63	0.64	0.64	0.64	0.65	0.66	0.68	0.70	0.72	0.80	0.17
60–80	0.49	0.56	0.49	0.56	0.48	0.52	0.51	0.52	0.63	0.52	0.52	0.46	0.47	0.46	0.47	0.44	0.52	0.12
80–100	0.45	0.48	0.40	0.46	0.45	0.47	0.49	0.44	0.46	0.44	0.46	0.39	0.43	0.51	0.41	0.40	0.45	0.12

Samples were taken from the Cerrado native vegetation (NV) on all three sampling dates, and in 2013 from all plots. In 2009 samples were taken from all high fertility plots, and in 2001 from all pastures and high fertility plots of ICL and continuous cropping

[a] LSD values represent the least significant differences between N concentrations (means of four replicates) in the same depth interval

(Fig. 1a, b). Earlier reports from Sisti et al. (2004) and Jantalia et al. (2007) indicated that below 40 cm depth in Oxisols there are no significant impacts on soil bulk density of traction of agricultural machinery, whether no-till planters, ploughs or harvesters.

Soil C and N concentrations

In all treatments the concentrations of total C and N decreased down the profile (Tables 4, 5), which is a characteristic of most soils under natural vegetation, pastures and no till agriculture (e.g. Bernoux et al. 2002; Groppo et al. 2015), owing to the rapid decline in the mass of crop roots with depth (e.g. Venke Filho et al. 2004; Caires et al. 2008). In an earlier sampling of this same field experiment in 2004 (Marchão et al. 2009) soil C and N concentrations were not observed to decrease with depth to 30 cm but ranged from 17 to 25 g C kg soil^{-1} and between 1.2 and 1.8 g N kg soil^{-1} in the 0–2, 2–5, 5–10, 10–20 and 20–30 cm depth intervals. The soil samples were analysed for total C and N using near infra-red spectroscopy (NIRS) and as no consistent decreases in soil C and N content with depth were observed, we assume that these C and N concentrations are incorrect, even though the NIRS results were calibrated with that of 92 samples analysed with an automatic CN analyser.

As has been observed before in the Cerrado region, the surface 10 cm of soil under the NV is high in C and this has been attributed to the slow decomposition of recalcitrant root residues of the NV (Roscoe et al. 2001). The soil under many treatments showed a tendency to have higher concentrations of C and N than under the NV of the Cerrado especially in the 10–40 cm depth intervals (Tables 4, 5).

N ratios

The C:N ratio of the soil samples from the area of NV ranged from means of 16.6 in the surface layer to between 21.9 and 23.1 in the depth intervals below 30 cm, which was generally above the ones observed for the respective soil layers, as an average of all treatments, 22 years after land use change (Table 6). It might be expected that under pure grass pastures, where plant litter and roots have high C:N ratios, that the surface layers of the soil might have high C:N ratios. However root biomass decreases (e.g. Holanda et al. 1998; de Castro and Kauffman 1998; Jobbágy and Jackson 2000) with depth along with decreased soil microbial activity (Babujia et al. 2010; Oliveira et al. 2004) such that most soil organic matter (SOM) is generally well humified ("heavy fraction") showing C:N ratios of less than 12 (Kirkby et al. 2011). Thus it does not seem credible that at deeper levels in the soil profile that (SOM) should show higher C:N ratios than at the surface.

Table 6 Mean C:N ratio for the soil layers sampled in 2013 from the native vegetation (Cerrado) areas and from the plots of the experiment

Depth	C:N ratio	
	Cerrado	Experiment
0–5	16.84 ± 0.93[a]	15.55 ± 0.31
5–10	17.10 ± 1.08	16.78 ± 0.19
10–20	19.02 ± 2.39	17.35 ± 0.24
20–30	19.37 ± 1.81	17.97 ± 0.21
30–40	21.20 ± 1.93	18.38 ± 0.25
40–60	21.95 ± 2.27	19.09 ± 0.41
60–80	23.10 ± 2.61	19.91 ± 0.32
80–100	21.37 ± 1.44	19.77 ± 0.32
Mean	19.99	18.10

[a] Standard error of the means

Such high C:N ratios have been observed in other regions of the Cerrado. Corbeels et al. (2006) found mean values of 18.1 for the 0–20 cm depth interval under 45 fields cropped under no-till management in the districts of Rio Verde (17°47′S, 51°55′W) and Montividiu (17°24′S, 51°14′W) in the south-eastern part of the Goiás state, some 400 km south west of Planaltina. In the same region under NV (4 sites) and pastures (2 sites) they registered mean C:N ratios of 20.6 and 23.2, respectively. The authors did not comment on these high C:N values.

However, other studies on Oxisols in the southern region of Brazil the C:N ratios down the profile to 100 cm were found to be close to 12 (e.g. Sisti et al. 2004; Diekow et al. 2005) and similar values were found under an Acrisol (Machado Pinheiro et al. 2010) and an Ultisol (Tarré et al. 2001) in more northerly regions of the Atlantic forest. Kirkby et al. (2011) made a very comprehensive survey on 598 soils (depth intervals not given) from all over the world outside of Australia and 59 Australian soils (0–15 cm) and arrived at a mean C:N value of between 11.5 and 12. We attribute the much higher C:N ratios found under this experiment and the neighbouring NV to the presence of charcoal/black carbon as the Cerrado region has a history of regular fires (Coutinho 1990) while the Atlantic forest was cleared with axe or chainsaws and the detritus only burned once (Dean 1995).

Jantalia et al. (2007), working on the Embrapa Cerrados field station at a site only 3 km from the long-term experiment featured in this present study, found C:N ratios in soil sampled at 8 depth intervals to 100 cm under NV and cropped land between 18 and 23. Initial studies with a stereomicroscope revealed

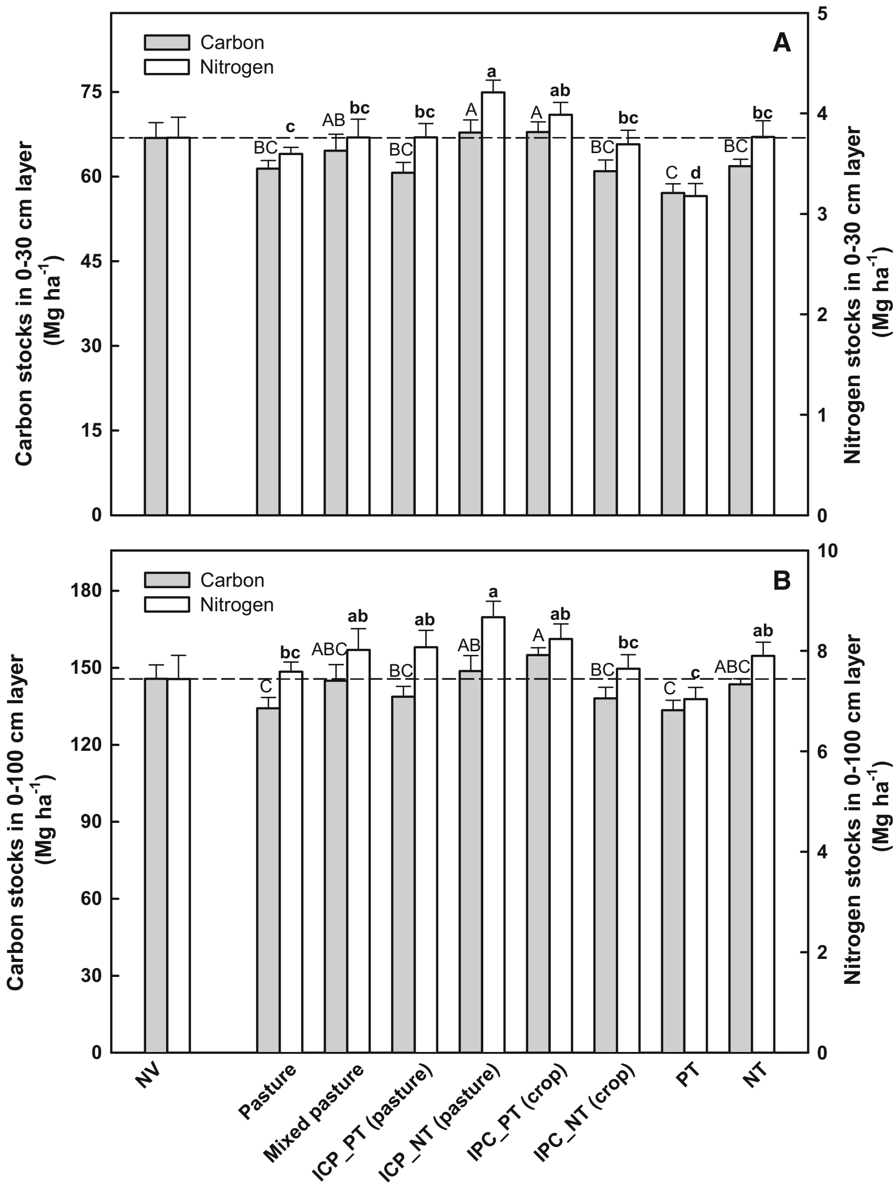
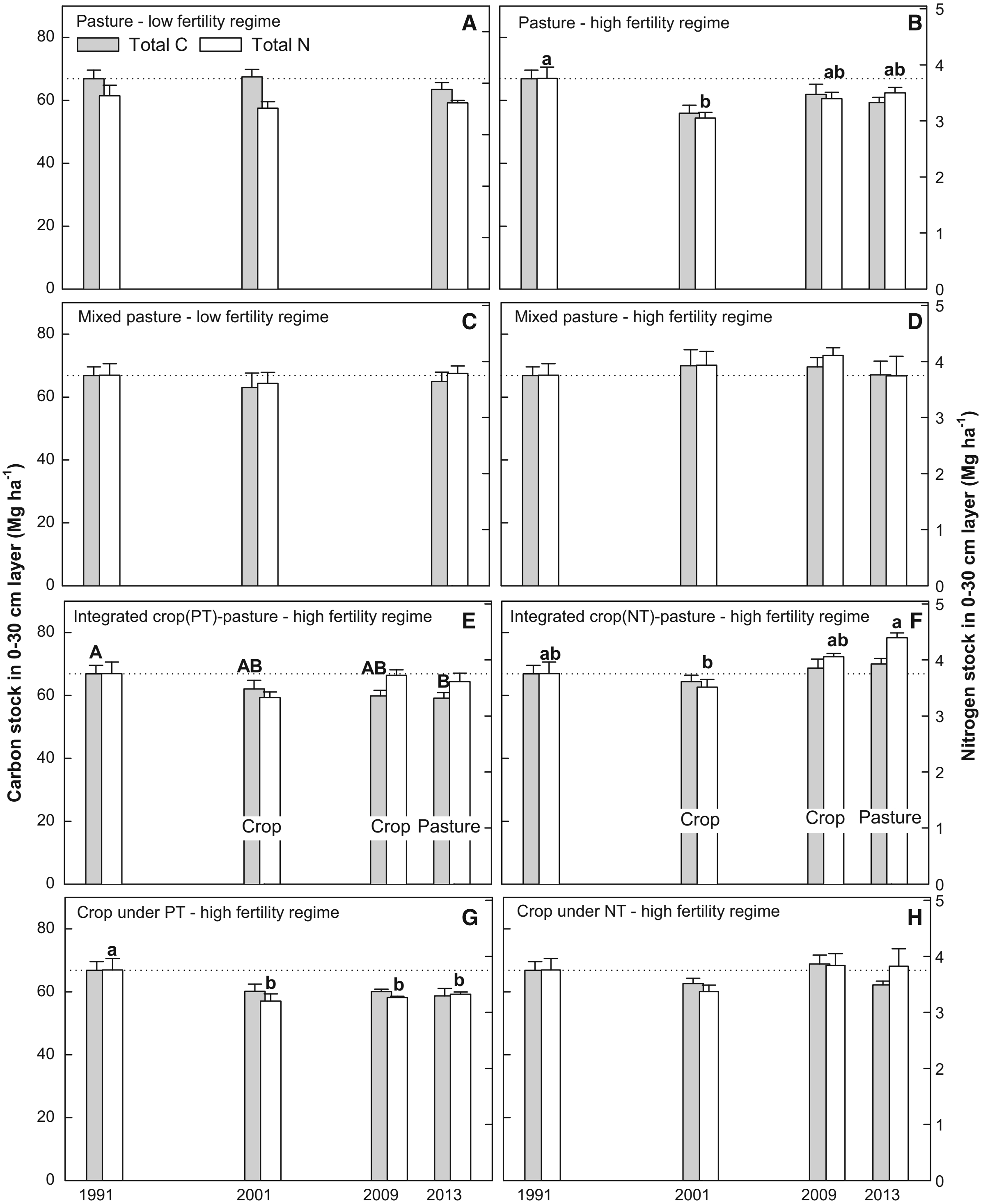


Fig. 2 The stocks of soil C and N under each land-use treatment (means of F1 and F2). The soil C and N stocks were corrected for an equal mass of soil under the native vegetation to 30 cm (**a**) or to 100 cm (**b**). *Horizontal lines* indicate the soil C and N stocks under the native vegetation. *Bars* representing stocks of total C or N stocks topped by the same *upper-case letters* and *lower case letters* respectively, are not significantly different at $P < 0.05$ (Student LSD test)



Pasture - low fertility regime
A
Total C
Total N
Pasture - high fertility regime
B
a
b
ab
ab
Mixed pasture - low fertility regime
C
Mixed pasture - high fertility regime
D
Integrated crop(PT)-pasture - high fertility regime
E
A
AB
AB
B
Crop
Crop
Pasture
Integrated crop(NT)-pasture - high fertility regime
F
ab
b
ab
a
Crop
Crop
Pasture
Crop under PT - high fertility regime
G
a
b
b
b
Crop under NT - high fertility regime
H
Carbon stock in 0-30 cm layer (Mg ha-1)
Nitrogen stock in 0-30 cm layer (Mg ha-1)
1991
2001
2009
2013
1991
2001
2009
2013

◀**Fig. 3** Changes in stocks of soil C and N to a depth of 30 cm over a period of 22 years from pure grass and grass/legume pastures at the lower (F1) and higher fertility (F2) regimes, under the ILP treatments (both F2) under no-till (NT) and conventional plough tillage (PT) and the continuous cropping under NT and PT. These stocks were compared with those existing under the undisturbed native vegetation in 2013, which were considered to represent the stocks present in the whole area when the experiment was first established in 1991. *Horizontal lines* indicate the soil C and N stocks under the native vegetation. *Bars* representing stocks of total C or N stocks topped by the same *upper-case letters* and *lower case letters* respectively, are not significantly different at $P < 0.05$ (Student LSD test)

large numbers of particles of what appeared to be charcoal at all depth intervals. Using a technique based on oxidation with 30% hydrogen peroxide (Jackson 1958) similar to that of Kurth et al. (2006), Jantalia et al. (2007) concluded that at least 30% of the soil C was charcoal. We did not have the resources available to analyse charcoal in the soil samples from this experiment. However, as in the experiment plots no fires occurred, any change in soil C stocks would be due to changes in SOC and not charcoal or black carbon.

Soil C and N stocks

In this experiment, all treatments of pastures, continuous cropping and ICL were cultivated at two different fertility levels: F1 and F2. The fertilizers and lime added to each treatment are given in Table 3. Only in 2013 were soil samples taken from all treatments and it was found that there were no statistical differences ($P > 0.05$—data not shown) between the different fertility levels for the stocks of soil C or N stocks to 30 or to 100 cm (adjusted for the same mass of soil under the NV to these depths). For this reason, the individual means for F1 and F2 are not displayed. The native Cerrado area was divided into four plots, two at each longitudinal extremity of the experiment. However, as these NV plots were not randomised within the experimental design, no statistical comparison can be made between the C and N stocks under these plots and those of the main experiment. However, if it is assumed that these stocks of C and N represent those under the area used for the experiment, it appears that the total C and N stocks under the grass-only pasture did not increase over the 22 years period. This is in contrast to other reports in the Cerrado (Corazza et al. 1999; Braz et al. 2013) and other regions of Brazil (Moraes et al. 1996; Koutika et al. 1997).

There was a tendency for the presence of legumes in the pasture to increase soil C and N stocks compared to the grass-alone pastures, but these differences were not significant at $P < 0.05$ to either 30 or 100 cm depth (Fig. 2). Fisher et al. (1994) reported highly significant increases in C stocks due to the presence of legumes in a *Brachiaria humidicola* pasture in the eastern savanna (Llanos Orientales) of Colombia. A positive effect of a forage legume in a pasture of *B. humidicola* on the accumulation of soil C and N also was reported by Tarré et al. (2001) in study performed in the coastal Atlantic forest region of the South of Bahia, Brazil.

The most significant differences in soil N stocks were found in the comparison of NT and PT management of continuous cropping (Fig. 2). To a depth of 30 or 100 cm, N stocks were significantly ($P < 0.05$) higher under NT than under PT. The same tendency was apparent for the C stocks but the differences were not statistically different at $P < 0.05$. There are many reports in Brazil that C stocks under NT increase after years of conventional PT and, while the first studies investigated stocks to less than 30 cm (e.g. Bayer and Mielniczuk 1997), subsequent studies have confirmed this to depths well below the plough layer both in the southern region (e.g. Sisti et al. 2004; Diekow et al. 2005) and in the Cerrado (Zinn et al. 2005; Jantalia et al. 2007). These results are consistent with conclusions based on recently published results taken from multiple sites in the Cerrado region (Corbeels et al. 2016) and elsewhere in Brazil (Assad et al. 2013): continuous cropping under NT may increase SOC stocks on land that has been under PT for several years, but the SOC stocks under NT cropping rarely exceed those under the original native vegetation.

The results of the C and N stocks under the ICL systems are unexpected. The C stocks to 30 cm were highest under two systems: the ICP managed under NT (ICL-NT pasture) sampled in the pasture phase and that managed under PT sampled in the crop phase (ILC-PT crop) (Fig. 2a). These C stocks were significantly ($P < 0.05$) higher than those under the ICL system managed under PT and sampled in the pasture phase (ICL-PT pasture) or those under the ICL system managed under NT and sampled in the cropping phase (ILC-NT crop). Considering the results from continuous cropping treatments, it is to be expected that management under NT would result in higher stocks of soil C and N than ICP managed under conventional

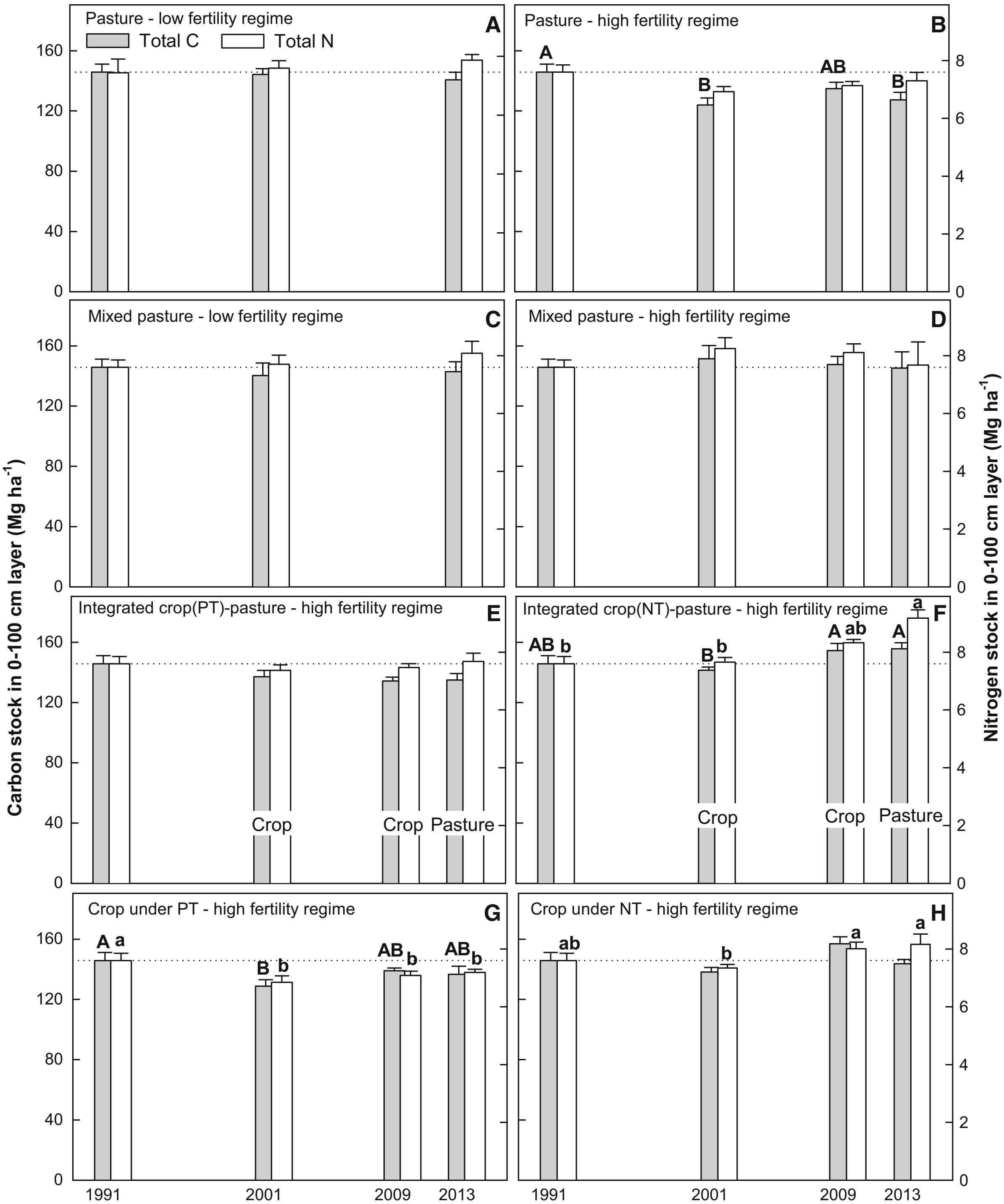


Fig. 4 Changes in stocks of soil C and N to a depth of 100 cm over a period of 22 years from pure grass and grass legume pastures at the lower (F1) and higher fertility (F2) regimes, under the ILP treatments (both F2) under no-till (NT) and conventional plough tillage (PT) and the continuous cropping under NT and PT. These stocks were compared with those existing under the undisturbed native vegetation in 2013, which were considered to represent the stocks present in the whole area when the experiment was first established in 1991. *Horizontal lines* indicate the soil C and N stocks under the native vegetation. *Bars* representing stocks of total C or N stocks topped by the same *upper-case letters* and *lower case letters* respectively, are not significantly different at $P < 0.05$ (Student LSD test)

PT. This trend is not apparent for any of the ICL systems managed under PT or NT and neither were there any consistent differences between the systems sampled during the pasture or cropping phase. In general terms it is apparent that ICL conserved stocks of C and N in the soil, compared to conventional PT continuous cropping, but the differences in C or N stocks were not always statistically significant at $P < 0.05$.

The soil under the plots/paddocks of all higher fertility (F2) treatments (except the ICL starting with cropping) were sampled to 100 cm depth three times during the experiment, in 2001, 2009 and 2013. All samples were analysed shortly after sampling such that the same instrument or method was not used for all three sets of samples. Hence, as explained in the Materials and methods, all soil samples from 2001 to 2009 to a depth of 30 cm were reanalysed simultaneously for total C and N along with the 2013 samples on the same Costech analyser, coupled or not, to the Finnigan Delta-V mass spectrometer.

From these results were calculated the stocks of total C and N for the soils under these F2 treatments to depths of 0–30 cm (Fig. 3) and 0–100 cm (Fig. 4). Once again, the stocks of C and N in 1991, before the installation of the experiment were assumed to be equal to the stocks in the NV areas in 2013. The most striking aspect of the results is that in most treatments the changes in soil C or N stocks did not change significantly ($P < 0.05$) with time. In the pastures there were no significant changes in stocks to a depth of 30 cm of C or N except for the high fertility treatment of the grass-alone pasture. Here there was a significant decrease ($P < 0.05$) in N stock in the period 1991–2005 when measured to 30 cm and this decrease in SOM was confirmed at the same level of significance for the C stock to a depth of 100 cm in 2001 and at the final sampling in 2013.

There was also a significant ($P < 0.05$) fall in the C stocks to 30 cm in the ICL system under the high fertility regime managed under PT but this decrease was not significant for the estimate of the stocks of C or N to 100 cm. On the other hand, there was a significant increase ($P < 0.05$) in N stocks to 30 cm and the N and C stocks to 100 cm depth in the ICL system under the high fertility regime managed under NT. These results are consistent with the hypothesis that ICL systems managed under NT should help to accumulate soil carbon. This was not apparent in the comparison of all the samples taken at the final sampling 2013 (Fig. 2) probably because the effects were attenuated in the low fertility regime.

The time series data also confirm the benefit of the use of NT in conserving soil C and N under continuous cropping. Soil N stocks (0–30 cm) decreased significantly ($P < 0.05$) under PT (high fertility regime) and this was also significant for N stocks to 100 cm. N stocks (0–100 cm) under NT also significantly ($P < 0.05$) increased from 2001 to 2013.

Separation of C derived from native vegetation or C_4 grasses

Soil samples taken to 100 cm depth under the NV and under all four pastures treatments (both F1 and F2) in 2001 and 2013 were analysed, not only for C and N concentration, but also for ^{13}C natural abundance (Fig. 5). As has been observed before in the Cerrado region, the ^{13}C abundance under the NV becomes less negative with depth, which indicates that in more recent times there has been an increase in the proportions of trees/shrubs (C_3 plants) in the natural vegetation (Roscoe et al. 2000; Jantalia et al. 2007; Braz et al. 2013). In 2001 there was a large and significant ($P < 0.05$) difference in ^{13}C abundance of the soil under the NV and that under the pure grass pastures down to a depth of 40 cm, but no difference between the ^{13}C abundance of the soil under the pastures and the NV below 80 cm (Fig. 5a). These differences indicated that there was considerable deposition of C_4 grass residues down to a depth of 40 cm and some C_4–C deposition as far as 80 cm depth after 10 years.

However, after a further 12 years of pasture the ^{13}C abundance showed that there was an increase in the deposition of C_4 grass residues down the whole of the profile to 100 cm depth (Fig. 5b). These results indicate that in the Cerrado region, with its long (>5 month) dry season, roots of grasses such as *Brachiaria brizantha* penetrate to at least 1 m depth and deposit significant quantities of C_4 carbon. Some evidence for this was also found in the study of Braz et al. (2013), but being a study of chronosequences, the evidence was less reliable. Evidence for an input of deep carbon by *Brachiaria* spp. in a savannah biome (Llanos Orientales of Colombia) was first suggested by the results of Fisher et al. (1994). In contrast, in the South of Bahia which has no marked dry season, Tarré et al. (2001) found no evidence for deposition of C_4–C

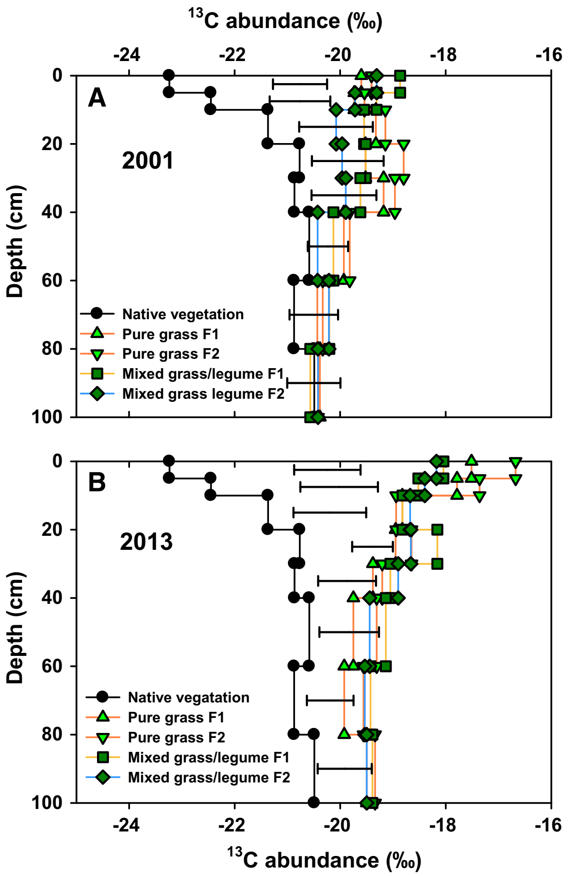


Fig. 5 The ^{13}C natural abundance of soil samples taken to a depth of 100 cm in eight depth intervals from the pure grass and mixed grass/legume pastures under F1 and F2 fertility regimes in 2001 (**a**) and 2013 (**b**). *Error bars* represent the least significant difference at $P < 0.05$ (Student LSD test) between means of four replicates

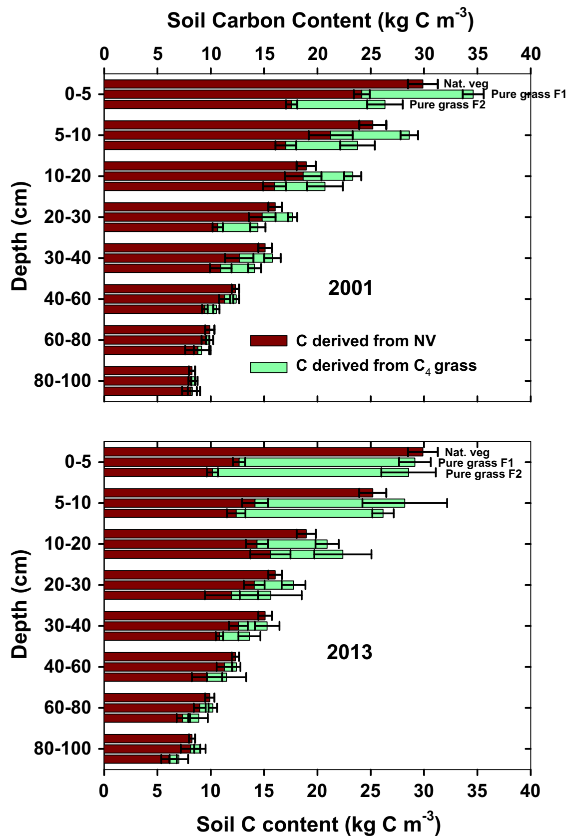


Fig. 6 Carbon (kg C m^{-3}) derived from the native Cerrado vegetation (NV) and from the C_4 grass in the pure grass pasture at the low (F1) and high (F2) fertility regimes. From *top* to *bottom*, the *three bars* in each group represent the C under the natural vegetation, pure grass at F1 and pure grass at F2. *Error bars* represent standard errors of the means of the C derived from the NV and the C derived from the C_4 grass

from *B. humidicola* below 40 cm depth after 9 years of pasture establishment on an area cleared of Atlantic forest vegetation.

In the case of the mixed grass/legume pastures, any input of legume-derived C would cause the ^{13}C abundance to become more negative due to inputs of C_3 carbon at approximately −27 to −28‰ (Tarré et al. 2001). This effect is clearly visible in the top 10 cm of the soil after 22 years of pasture (Fig. 5b). However, at depths below 40 cm in the soil, there was little difference between the ^{13}C abundance of the mixed grass/legume pastures and the pure grass pastures, which suggests that C_4 carbon derived from the grass roots was being deposited at depth but that there was no appreciable contribution of legume root-C deep in the profile.

Using the ^{13}C abundance and C concentration data it was possible to calculate the proportion of C derived from the NV and from the C_4 grass down the profile (Fig. 6) in the grass-alone treatments. The stocks of the C derived from the NV and the *Brachiaria* corrected to the mass of soil under the NV were calculated for the years 2001 and 2013 for both fertility treatments and compared with the original stock of total C under the NV.

The data show that from 2001 to 2013 in the 0–20 cm depth intervals, the amount of C derived from the NV decreased significantly ($P < 0.05$) (Fig. 5). This can only be explained by the fact that in the first 10 years since pasture establishment that the C derived from the original NV decomposed more rapidly in the higher fertility treatment. This decrease was from 62.5 to 41.0 Mg C ha^{-1} in the 0–30 cm depth interval compared to a decrease to only 51.2 Mg C ha^{-1} in the lower fertility treatment, a difference of 10.2 Mg C ha^{-1} (Fig. 7). This difference in the stock of remaining soil C derived from the NV to a depth of 100 cm after 10 years amounted to 20.4 Mg C ha^{-1}. The amount of C derived from the NV to a depth of 30 and to 100 cm was significantly lower ($P < 0.05$) in the higher fertility treatment (F2) than in F1 in 2001. This trend continued to be apparent for the samples taken in 2013, but the difference was not significantly different at $P < 0.05$. The quantities of C derived from the C_4 grasses were very similar between the fertility treatments, 22.8 and 22.4 Mg C ha^{-1} for F1 and F2,

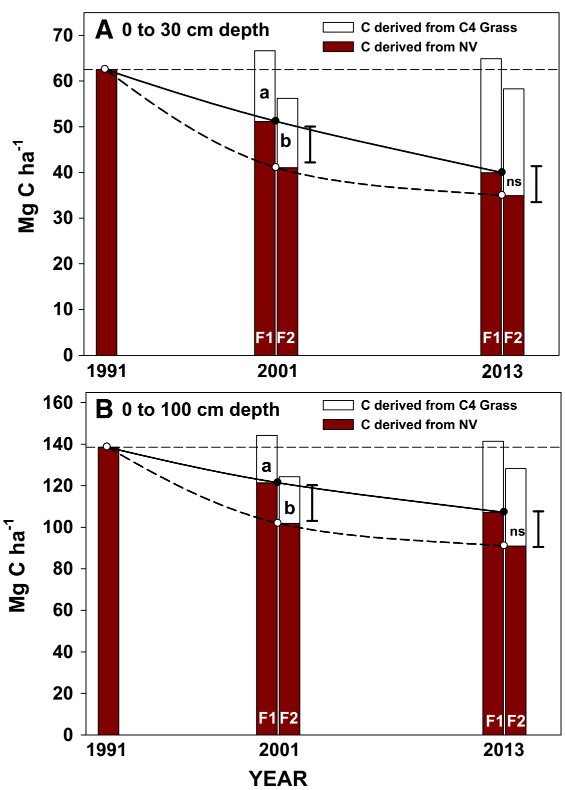


Fig. 7 Stocks of total carbon derived from native vegetation (NV) and the C_4 grass corrected for an equal mass of soil under the NV to 30 (**a**) and to 100 cm (**b**) under the pure grass pastures in the low (F1) and high fertility (F2) treatments. The stock of C derived from the NV immediately before the establishment of the experiment in 1991 were assumed to be equal to the stock present under the NV in 2013. The error bars represent the least significant difference at $P < 0.05$ (Student LSD test), and *different letters* and ns (non-significant) all refer to the differences in the stock of C derived from the NV between the F1 and F2 treatments

respectively, to a depth of 100 cm in 2001, and in 2013 these values were, respectively, 34.3 and 37.2 Mg C ha^{-1}.

These results were unexpected as it was thought that the higher fertility would promote more root growth and hence greater accumulation of soil C. However, the amount of soil C derived from the C_4 grasses was not significantly affected by the amounts of fertilizers added to the pastures, but increased the decomposition rate of the C derived from the NV. It seems established that soils under higher fertilizer inputs show higher microbial biomass (Geisseler and Scow 2014). This would explain the higher rate of soil C respiration and hence C loss, observed in this study. However, in a study at the same field station by de Castro Lopes et al. (2013) higher inputs of P fertilizer which resulted in higher available (Mehlich) P levels were positively correlated with crop yields, microbial biomass-C and the concentration of soil organic carbon (SOC) to 10 cm depth. However, whether the original SOM derived from the NV decomposed more rapidly under higher fertilizer inputs could not be determined.

We can find no other reference where a C_4 crop was grown for many years on a soil predominantly or partially originating from C_3 vegetation and the soil samples subjected to ^{13}C abundance analysis. The results of our study imply that in some circumstances increasing fertiliser additions may lead to higher rates of SOM decomposition and this may not always be compensated for by increased deposition of crop-root derived carbon.

Conclusions

The results of this long-term study confirm many earlier studies that continuous cropping under conventional plough tillage leads to greater losses of SOC than when managed under no-till. The results also confirm that the presence of a legume in the pastures has a positive effect on soil C accumulation or conservation. With regard to integrated crop pasture (ICL) systems, the highest carbon stocks were found under these systems, but one treatment where no-till was used throughout to install all crops and pastures, the C stocks were inexplicably lower than nearly all other treatments. The effect of increasing inputs of lime and N, P and K fertilizers on soil C stocks under continuous C_4-grass pastures was contrary to expectations, and showed that soil C stocks showed a strong tendency to decrease after 10 years of establishment. Using ^{13}C abundance it was found that the amount of soil C derived from the native vegetation was significantly lower in the higher fertility treatment while the improved fertility had no significant effect on the quantity of C derived from the C_4 grass. In other words, the mineral fertilizers stimulated the decomposition of the C derived from the SOM present in the NV before the experiment was established.

The important lesson learned from this long-term experiment is that conversion of the Cerrado to cropping, or ICL systems, under NT may not necessarily lead to large gains in soil C. The largest increase in soil C over 22 years was approximately 16 Mg C ha^{-1}, and in this tropical climate it is likely that the new equilibrium of the rates of C deposition and decomposition have been reached and further C accumulation will not occur (West and Six 2007; Corbeels et al. 2016).

The results suggest that the main benefit of intensifying beef cattle pasture systems in the Cerrado region by use of fertilizers and improved forages, or introducing integrated systems of crop/livestock, does not lie in removing atmospheric CO_2 and storing it in soil, although intensification of the systems may reduce emissions of other GHGs per kg of animal product (Cardoso et al. 2016).

Acknowledgements The authors would like to express their gratitude to all the field workers and technicians at Embrapa Cerrados who have maintained this experiment since 1991. The running of the experiment at Embrapa Cerrados was almost solely funded by different projects of Embrapa. The analyses were funded by Embrapa, the Brazilian National Research Council (CNPq) and the Rio State Research Foundation (FAPERJ). The authors SACSA and JMS, gratefully acknowledge their post-doctoral and PhD fellowships, respectively, and the authors CPJ, BJRA, SU and RMB grants and fellowships from CNPq and FAPERJ. The diligent work of Renato Moutinho da Rocha in the operation of the total CN analysers and the isotope-ratio mass spectrometers is gratefully acknowledged along with that of the team of the agricultural chemistry laboratory for Kjeldahl and soil fertility analyses.

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