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2 3	Fluxes of CO ₂ above a sugarcane plantation in Brazil
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27	Abstract
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29	Fluxes of CO ₂ were measured above a sugarcane plantation using the eddy-covariance
30	method covering two growth cycles, representing the second and third re-growth
31	(ratoons) harvested with stubble burning. The total net ecosystem exchange (NEE) in
32	the first cycle (second ratoon, 393 days long) was -1964±44 g C m ⁻² ; the gross
33	ecosystem productivity (GEP) was 3612±46 g C $\text{m}^{\text{-}2}$ and the ecosystem respiration (R_E)
34	was 1648±14 g C m ⁻² . The NEE and GEP totals in the second cycle (third ratoon, 374
35	days long) decreased 51% and 25%, respectively and $R_{\rm E}$ increased 7%. Accounting for
36	the carbon emitted during biomass burning and the removal of stalks at harvest, net
37	ecosystem carbon balance (NECB) totals were $102\pm130~g~C~m^{-2}$ and $403\pm84~g~C~m^{-2}$ in
38	each cycle respectively. Thus the sugarcane agrosystem was approximately carbon
39	neutral in the second ratoon. Yield in stalks fresh weight (SFW) attained the regional
40	average (8.3 kg SFW $\mbox{m}^{\mbox{-}2}\mbox{)}.$ Although it was a carbon source to the atmosphere, observed
41	productivity (6.2 kg SFW m ⁻²) of the third ratoon was 19% lower than the regional
42	average due to the lower water availability observed during the initial 120 days of re-
43	growth. However, the overall water use efficiency (WUE) achieved in the first cycle
44	(4.3 g C kg ⁻¹ H ₂ O) decreased only 5% in the second cycle.
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59	Key words: CO ₂ fluxes; sugarcane; respiration; eddy covariance; biomass; soil water
60	deficits

1-Introduction

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The increasing global demand for sustainable energy sources has led to more agricultural land being used for biofuel production. There are many concerns about the environmental impacts of growing more biofuels (Martinelli and Filoso, 2008; 66 Martinelli et al., 2010; Lisboa et al., 2011; Loarie et al., 2011), including their impact on the carbon cycle (Verma et al., 2005; Beringer et al., 2011; Zenone et al., 2011). The greenhouse gas (GHG) balance of an energy crop will depend on the crop type, climate, soil and management (Don et al., 2012), and the net carbon balance of energy crop 70 cultivation may increase GHG emissions if not adequately managed (Beringer et al., 2011). The same is true when residues from agriculture and forestry are used as biofuel. Although it is often assumed that the carbon input and output of croplands are in equilibrium (Moors et al., 2010), this crude simplification of the production cycle can produce misleading results (see Moureaux et al., 2008; Aubinet et al., 2009; Béziat et al., 2009; Kutsch et al., 2010; Ceschia et al., 2010; Routa et al., 2011; Zeri et al., 2011). 76 The sugarcane in Brazil is grown almost entirely under rain-fed conditions and preharvest burning is practiced in approximately 60% of the total area (Pinheiro et al., 2010). After the first year of establishment the yields of the re-growth from the stubble (ration crop) decrease at an average rate of approximately 10% per year (Cabral et al., 2012). After four successive harvests the yield falls to 7.0 kg m⁻² and the plantation is re-established (Macedo et al., 2008). In 2009, Brazil produced 696 Tg of sugarcane, from an area of 86,800 km², with 54% of this being in the southeastern state of São Paulo (Brasil, 2010). According to the expected domestic and international demands, over the next 10 years sugar and bioethanol production should increase by 49% and 127%, respectively. Over the same period the planted area is expected to increase by

49% (Brasil, 2010), i.e., to some 129,000 km². These figures imply that the area 86 87 available for expansion is not limiting; and indeed it has been estimated that the total area available is 647,000 km², with approximately 30% classified as highly productive 88 89 (Manzatto et al., 2009). However, based on the last 20 years' mean annual production, 90 which exhibited coefficients of variation ranging from 5 to 12% between regions (Marin 91 et al., 2011), considerable challenges must be overcome to achieve these forecasts. 92 Although part of this variation can be attributed to growing varieties not well adapted to 93 soil and climate, and to low levels of plant nutrition, the importance of adequate water 94 supply needs to be addressed. The often low water availability resulting from the inter-95 annual variability of climate in the southeast of Brazil (Dufek and Ambrizzi, 2008; 96 Marengo et al., 2009; Cabral et al., 2011) and particularly in São Paulo State is an 97 important factor influencing plant growth (van den Berg et al., 2000; Cabral et al., 2010; 98 Cabral et al., 2012) and the consequent carbon budgets. 99 The CO₂ fluxes obtained by the eddy-covariance method (Aubinet et al., 2000; 100 Baldocchi, 2003) above a vegetated surface represent the net CO₂ ecosystem exchange 101 (NEE), whose sign indicates whether the system is storing (negative) or releasing 102 (positive) carbon (Valentini et al., 2000; Law et al., 2002; Leuning et al., 2005) and 103 represents the balance between the fluxes given by the gross ecosystem productivity 104 (GEP) and ecosystem respiration (R_E). The components of R_E ($=R_H+R_A$) result from the 105 decomposition of organic matter, the heterotrophic respiration (R_H) and the autotrophic 106 component (R_A) associated with the biomass growth and maintenance (Chapin et al., 107 2006). The net primary productivity (NPP=GEP-R_A) can be approximately assessed 108 from the measurements of the main biomass components (Smith et al., 2010) and differs 109 from NEE (=GEP- R_F) by the heterotrophic respiration (Falge et al., 2002). The net 110 ecosystem carbon balance (NECB) is defined as the sum of NEE and the carbon losses

by non-respiratory processes such as run-off, pre-harvest burning and harvest (Anthoni et al., 2004; Verma et al., 2005), as well as the carbon inputs as fertilizer and seeds (Smith et al, 2010).

The objectives of this work were to observe the seasonal dynamics in the net ecosystem exchange in a representative sugarcane agrosystem and to characterize how its constituents, GEP and R_E, respond to environmental factors. The establishment of the net ecosystem carbon balance (NECB) and the water use efficiency (WUE) of the sugarcane crop, will contribute to reduction of the large uncertainty in the carbon balance of tropical C4 ecosystems.

2-Methods

2.1-Site

The sugarcane (*Saccharum* spp hybrids) plantation is located in Luiz Antonio municipality in São Paulo State, Brazil (21° 38′ S, 47° 47′ W) at 552 m altitude.

The maximum rain falls in December (274±97 mm month⁻¹) and the minimum in July and August (27±34 mm month⁻¹); the mean annual precipitation (for years 1971 to 2007) and its standard deviation are 1517±274 mm. The mean temperatures in January and July are 24 °C and 19 °C, respectively, and the annual mean is 22 °C. The mean dry bulk density of the sandy soil (Typic Haplustox) down to 2.6 m depth is 1500 kg m⁻³, and the available soil water in the first meter is 136 mm. The area (> 400 ha) exhibited a small slope of less than 2%. During unstable conditions, flux footprint calculations (see Hsieh et al., 2000) indicated that >90% of the measured fluxes emanated from the uniform crop of sugarcane under study. The distance between planting rows was 1.4 m

and the maximum canopy height achieved was approximately 5 m (further details are given by Cabral et al., 2012; Cuadra et al., 2012).

The measurements reported here began just after the second harvest (first ratoon), which occurred in 13/04/2005 (day 103) and extended to the harvest in 20/05/2007 (day 140) covering two cycles which represented the second (ended in 11/05/2006, day 131) and third re-growth from the stubble (ratoons). The pre-harvest practices included the burning and the aerial spraying of chemical ripeners to increase the sucrose accumulation in the sugarcane stalks (see Inman-Bamber et al., 2008; Dalley and Richard, 2010); spraying occurred in 22/03/2006 and 01/04/2007, respectively. One week after both harvests the area received 400 kg ha⁻¹ of limestone (CaCO₃), which was equivalent to 4.8 g C m⁻² supposing it was in the form of calcite; and urea-based fertilizer representing 56 kg N ha⁻¹ and 150 kg K₂O ha⁻¹ of potassium. The planted varieties (SP81-3250, SP83-2847 and RB86-7515), the soil and achieved yields were representative of southern Brazilian conditions (see Marin et al., 2011; Cabral et al., 2012).

2.2-Instrumentation

The sonic anemometer (R2, Gill Inst., Lymington, Hampshire, UK) and the air intake of the closed-path infrared gas analyzer (IRGA, LI6262, Li-Cor Biosciences, Lincoln, NE, USA) were deployed on top of a lattice tower at 9 m height. The air was continuously pumped (5 L min⁻¹, UN89-KNF Neuberger GmbH, Freiburg, Germany) to the IRGA through a 10 m length of heated polyethylene tubing (4 mm I.D.) and two 1.0 µm poresize membrane filters (Gelman Acro 50, Pall Corporation, Port Washington, NY, USA). The reference cell of the IRGA was purged with nitrogen, and calibrations were

performed manually every two weeks. A datalogger (CR1000, Campbell SI, Logan, UT, USA) recorded all the raw data (21 Hz) for post-processing.

The air temperature, humidity (HMP45, Vaisala, Helsinki, Finland) and rainfall (TB4, Hydrological Services Pty. Ltd., NSW, Australia) were measured at 6 m height; the net radiation (R_n), global radiation (R_g) and photosynthetically active radiation (PAR) sensors (Kipp and Zonen, Delft, The Netherlands) were fixed at 7 m height on a horizontal boom 2.5 m away from the tower. The soil moisture content (SWC) was measured by 10 reflectometers (CS615, Campbell SI, Logan, UT, USA) installed vertically (0.3 m layers), down to 3 m depth. The soil heat flux was obtained from the mean of four plates (REBS, Seattle, WA, USA) installed within and between the planting rows, buried at 25 mm depth.

2.3-Data processing

 CO_2 flux (F_{CO_2}) was obtained from the covariances between the fluctuations (with respect to 30-minute block averages) of vertical wind speed (w) and CO_2 air concentration. The effects of sonic anemometer misalignment or topographically induced flow were accounted for with a double rotation forcing the mean vertical windspeed to zero (Kaimal and Finigan, 1994); the time lag between w and CO_2 was removed prior to the calculation of the covariances; this procedure also corrects for the sensor separation and phase-shift (see Ibrom et al., 2007). The half-hour averages were also discarded if the values were outside predefined realistic bounds (see Zeri et al., 2011) and filtered to remove periods of non-stationarity (Foken et al., 2004). During stable atmospheric conditions the friction velocity (U_*) filter was applied (Aubinet et al., 2000; Saleska et al., 2003; Reichstein et al., 2005). The spectral corrections

186 necessary to recover the high frequency flux loss (Moore, 1986) imposed by the closed-187 path system were derived using the low-pass filter technique (see Massman and Lee, 188 2002; Sakai et al., 2004; Ibrom et al., 2007; Mammarella et al., 2009), whereby the 189 estimated characteristic time (τ_s) of the assumed first order sensor response $([1+(2\pi f\tau_s)^2]^{-1}$, where f is the natural frequency) is applied to the measured cospectra of 190 heat fluxes in order to represent the observed degraded spectra of the F_{CO}, fluxes and 191 192 obtain the necessary correction factor (see Aubinet et al., 2000). The CO_2 storage (S_{CO_2}) in the air column below the eddy-covariance system necessary 193 194 to calculate the net ecosystem exchange (NEE = F_{CO_2} + S_{CO_2}) was estimated from the 195 time variation of air CO₂ density measured at 9 m height by the IRGA, with the implicit 196 assumption that the CO₂ concentration does not change with height (see Flanagan et al., 197 2002; Anthoni et al., 2004; Verma et al., 2005; Béziat et al., 2009; Zeri et al., 2011). 198 This assumption is supported by the CO₂ profiles observed in maize reported by Santos 199 et al. (2011), obtained during turbulent conditions when the storage was considered 200 negligible and by Sakai et al. (2004) who measured the fluxes at a height of 20 m above 201 pasture and found that the storage fluctuated around zero for higher U*, although Saito 202 et al. (2005) did find for their particular site (paddy rice) instantaneous NEE errors as 203 large as 21% when using the single level to estimate the S_{CO_2} . 204 The missing daylight NEE fluxes were filled by the application of hyperbolic 205 relationships obtained between half-hourly NEE and PAR (Falge et al., 2001; 206 Reichstein et al., 2005) fitted over 15-day non-overlapping windows. When U_{*} was 207 below the estimated threshold (Goulden et al., 1996; Aubinet et al., 2000; Saleska et al., 208 2003) the NEE values were filled or replaced by the results of exponential curves 209 relating the ecosystem respiration (R_E) and air temperature for a range of soil water 210 content (see Reichstein et al., 2002).

The gross ecosystem productivity (GEP) was calculated as the difference between the ecosystem respiration (R_E) during daylight hours, obtained from the fitted exponential relationships between nighttime CO₂ fluxes (R_E) and air temperature (see Reichstein et al., 2002) and the observed NEE (=R_E-GEP), whose negative flux indicated a net downward transport of CO₂. The uncertainties associated with the eddy-covariance fluxes (see Moncrieff et al., 1996; Goulden et al., 1996) covered the errors due to the gap-filling models (Falge et al., 2001; Oren et al. 2006), the random errors (Hollinger and Richardson, 2005) and the selective systematic bias introduced by the NEE estimates under low turbulence conditions (Aurela et al., 2002; Papale et al., 2006; Béziat et al., 2009). The quality of CO₂ fluxes was assured by the energy balance closure (see Wilson et al., 2001; Culf et al., 2004; Leuning et al., 2005; Foken, 2008) shown by Cabral et al. (2012), who found a slope of 0.97 in the linear regression through the origin between the sum of turbulent fluxes of heat and water vapor versus the available energy. Thus the cospectral corrections (Moore, 1986) utilized were effective and the necessary conditions for the eddy-covariance method, such as an adequate fetch (Hsieh et al., 2000) of homogeneous surface, have been met (see Baldocchi, 2003). However the recent results of Frank et al. (2013) showed that the use of a non-orthogonal sonic anemometer (CSAT3, Campbell SI, Logan, UT, USA) can lead to 10% underestimation in w; if this is a general result it would be applicable to the sonic anemometer utilized

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here and this assumption must be reassessed.

The evapotranspiration fluxes (ET) necessary to calculate the water use efficiency

(WUE=GEP/ET) were measured simultaneously at the site; further details are given by

Cabral et al. (2012).

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2.4 – Sugarcane biomass

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The aboveground biomass (stalks; green and senesced leaves) was sampled in ten random plots, each 1 m along a planting line, which was equivalent to 1.4 m² of surface area. Samples were taken at approximately 20-day intervals and on each harvest day, i.e., 13/04/2005 (day 103); 11/05/2006 (day 131) and 20/05/2007 (day 140). Ten subsamples (10% of the fresh weight) were dried to constant weight in a forced ventilation oven (60 °C) and all them were analyzed for nitrogen concentration using the Kjeldahl digestion method (see Coombs et al., 1987) and for carbon by the dry combustion method (see Roberts et al., 1987). The mean specific leaf area was determined from the area and dry weight of leaf samples collected during both cycles for green (10.2 m² kg⁻¹) and senesced (9.6 m² kg⁻¹) leaves, and were used to obtain the leaf area indices of green (LAI_g) and dead (LAI_d, senesced) leaves respectively from the sampled dry biomass. The root-system estimate was derived from the root-to-shoot ratio (see Smith et al., 1999), based on the root and aboveground dry biomass data obtained in similar conditions reported by Faroni and Trivelin (2006), Otto et al. (2009) and Battie Laclau and Laclau (2009), whose mean root-to-shoot ratio was 0.122±0.056 over a range from 0.043 to 0.220. The estimated root loss during harvest of 17% was based on the work of Ball-Coelho et al. (1992). Error propagation was addressed by the accumulation principle (Aurela et al., 2002) assuming the fractions to be independent (see Béziat et al., 2009).

The amount of sugarcane residue remaining aboveground after harvest was assessed as the difference between the aboveground biomass samples (10 samples) before and after burning.

3 – Results and discussion

3.1 – Cospectral corrections, U_{*} filter and the ecosystem respiration estimates

The estimated first order sensor characteristic time (τ_s) was 0.35 s when the tubing of the closed-path system was new at the beginning of the measurements and 0.65 s two years later, at the end. However, applying these values of τ_s to the heat flux cospectra implied CO_2 flux losses varying from 10% (daytime) to 12% (nighttime) in agreement with Mammarella et al. (2009) who found a small difference in the flux correction for this range of τ_s . The corrections obtained here were similar to the results previously observed by Sakai et al. (2004) over grass but higher than the 4% obtained by Ibrom et al. (2007) and Mammarella et al. (2009), probably a consequence of the non-turbulent flow in the tubing we have utilized, as well as the greater contribution from eddies associated with higher attenuated frequencies above the sugarcane.

The plot of mean nighttime half-hourly NEE fluxes or the ecosystem respiration (R_E) versus the friction velocity (U_*) calculated from twenty intervals of U_* with the same number of points (Saleska et al., 2003; Reichstein et al., 2005), covering different stages of canopy development indicated that the nighttime NEE mean values were significantly lower (two-sample t test) for the median $U_* < 0.1 \text{ m s}^{-1}$ (p<0.05) and this U_* threshold was used to filter all nighttime fluxes (Reichstein et al., 2005). Values of

 $\rm U_*$ threshold as low as 0.1 m s⁻¹ were also found in stands of crops (maize, miscanthus and switchgrass) by Zeri et al. (2011), while values as low as 0.08 m s⁻¹ were reported by Sakai et al. (2004) over pasture and rice, and Aires et al. (2008) over a Mediterranean grassland site.

In order to gapfill the nighttime fluxes we derived exponential relationships (Van´t Hoff equation; see Lloyd and Taylor, 1994) between nighttime NEE data versus air temperature for a range of soil water contents (Reichstein et al., 2002; Cabral et al., 2011). The coefficients, given in Table 1, indicate sensitivities (Q₁₀) of the sugarcane system respiration to air temperature as low, as 1.2 representing the initial crop growth in the dry winter; but as high as 2.4, characteristic of the fully developed canopy in the wet summer. The intermediate curves contained data covering transient conditions spread over the whole period of measurement. The estimated increase in Q₁₀ implicitly represented the increase in biomass autotrophic respiration because the Q₁₀ dependence on soil moisture seasonality in fact reflected the sugarcane growth cycles and their different timing within seasons due to the distinct regrowth from stubble conditions (see Section 3.10.1 below). According to Mahecha et al. (2010) single-site studies of the intrinsic temperature dependence of respiration can be confounded by the factors seasonally co-varying with temperature.

<< Place Table 1 about here>>

3.2- Gap filling and the estimated errors

310 The amount of missing or rejected half-hourly fluxes due to instrument malfunction and 311 filtering was 20% during the first cycle and 18% in the second cycle, while 312 the number of periods when U_* was lower than the defined threshold (0.1 m s⁻¹) 313 accounted for 42% of all nighttime data in the first cycle and 33% in the second. 314 315 The estimated errors due to the gap filling were obtained by randomly replacing the 316 parameters of fitted models by perturbations based on the standard deviation of 317 parameter estimates (Oren et al., 2006) at each Monte Carlo iteration (1500 times). The 318 errors were calculated as the standard deviation of all cumulative fluxes generated (Falge et al., 2001; Moffat et al., 2007). The estimated uncertainties were ± 29 and ± 20 g 319 C m⁻² cycle⁻¹, respectively and the random error (Morgenstern et al., 2004; Richardson 320 and Hollinger, 2007) produced ±30 g C m⁻² cycle⁻¹. The selective systematic error 321 322 introduced by the replacement of the underestimated ecosystem respiration during calm 323 nights by the U_{*} filter (Moncrieff et al., 1996; Aurela et al., 2002; Papale et al., 2006) 324 was assessed by randomly sampling the observed nighttime data with replacement for 325 different U, thresholds (Anthoni et al., 2004; Papale et al., 2006; Béziat et al., 2009). The uncertainty of the U_* threshold detection resulted in errors of ± 14 g C m⁻² and ± 53 326 g C m⁻² for each cycle, respectively. Therefore the estimated uncertainties in NEE 327 328 measurements and the scatter in the fitted model results for each cycle, assuming the errors to be independent (Flanagan and Johnson, 2005), were ±44 and ±64 g C m⁻² 329 cycle⁻¹, respectively. 330 These values are above the limits (± 20 to ± 40 g C m⁻²) reported over different 331 agrosystems (Béziat et al., 2009; Zeri et al., 2011) and greater than the effect of gap 332 filling on the annual sums of NEE (±25 g C m⁻² yr⁻¹) given by Moffat et al. (2007), 333 334 although only based on forest sites.

We have applied the conventional steady state test which accordingly to Béziat et al. (2009) can lead to data losses around zero covariance. For our 30-minute NEE data set, 17% were within the threshold of $\pm 1.25~\mu mol~m^{-2}~s^{-1}$, however as reported by Béziat et al. (2009) the modified steady state test resulted in NEP differences lower than 3%. The R_E error was assessed from the nighttime U_* threshold error and from the associated errors of the fitted curves (SSE in Table 1) for the daylight hours utilizing the accumulation principle (Aurela et al., 2002), and resulted in $\pm 14~g$ C m⁻² and $\pm 53~g$ C m⁻² for each cycle, respectively.

3.3-The climate patterns

The air temperature shown in Fig. 1a ranged from 5 to 36 °C and the vapor pressure saturation deficit (VPD) from 0.1 to 5.0 kPa. The region is characterized by a relatively dry winter exhibiting low rainfall and consequently lower soil water content (Fig. 1b). This lack of soil water probably promoted some vegetation stress as the wilting point was nearly reached in September of both years. The total rainfall recorded in each cycle was 1194 and 1353 mm respectively, and although the first cycle total was lower than the long term average (1517±274 mm) the rainfall resulting from passing cold fronts in the winter of 2005 (203 mm) was greater than the total observed in the winter of 2006 (34 mm). The consequences of this inter-annual variability of rainfall on the water balance have already been reported by Cabral et al. (2012).

<< Place Figure 1 about here>>

The photosynthetically active radiation (PAR) and friction velocity are shown in Fig. 1c. The PAR fluxes show a clear seasonality whose maxima ranged from 1200 to 2300 $\,$ µmol quanta m $^{-2}$ s $^{-1}$ between winter and summer, respectively. However the intense summer rainfall observed in 2007 and the associated cloudy periods reduced the level of PAR fluxes by approximately 17%. U $_{*}$ also exhibited a seasonal variation with a maximum recorded between October and December due to the increased horizontal wind speed promoted by the regional atmospheric circulation, and the nighttime values observed during these months in 2006 were greater than 2005.

3.4 – Aboveground biomass and LAI

The measured aboveground biomass fractions and the green leaf area index are depicted in Fig. 2. The observed peak in green leaf biomass was 266 ± 22 g C m⁻² in the first cycle and 209 ± 12 g C m⁻² in the second cycle. However the peak biomass in dead leaves $(619\pm47$ g C m⁻²) and stalks $(1677\pm113$ g C m⁻²) measured in the first cycle decreased by approximately 43% in the second cycle and there was a delay in the canopy development of approximately two months – the maximum LAI_g (5.8) in the first cycle was achieved on 27/12/2005 (day 361), while in the second cycle (LAI_g=4.6) it was recorded on 14/02/2007 (day 45).

<< Place Figure 2 about here>>

The sugarcane cycle exhibits three distinct growth phases (Ramesh, 2000): the formative phase during the initial 150 days after harvest (DAH) when the germination and tillering are observed; the 'grand growth' (150-240 DAH) and maturity (240-360).

DAH) characterized by slower growth activity (Tejera et al., 2007). The decrease in yields, commonly observed in sugarcane rations (see Cabral et al., 2012), is mainly a consequence of the lower initial shoot population, whose unsprouted stubble promotes gaps in stubble rows (Shukla et al., 2009). The gaps in ratoons arise due to mechanical damage to stubble roots, from pests, diseases and nutritional limitations as well as the environmental growing conditions such as the level of soil water, and the timing and severity of early and mid-season water deficits (Robertson et al., 1999; Ramesh, 2000; Zhao et al., 2010). The dry matter partition in stalks and dead leaves during the formative phase in the first cycle, were 39% and 18% respectively; while in the second cycle the same biomass fractions represented 30% and 23% of the total dry matter, respectively. The reduced leaf area and the senescence of older leaves are typical dehydration avoidance mechanisms (Lopes et al., 2011). The early growth was dominated by the partitioning of dry mass into vegetative organs (Ramesh, 2000) particularly in the second cycle; however, as the crop advanced toward maturity, the same fraction of dry biomass, approximately 66%, was directed to the storage organs (stalks) in both cycles. Although the number of days after harvest (DAH) necessary to achieve the maximum LAI_g was practically the same in both cycles: 305 and 308 DAH, respectively, the leaf area duration (LAD; see Beadle, 1987; Chiariello et al., 1991) which represents the area under the LAI_g curve was 1144 days in the first cycle and 717 days in the second, implying in a decrease of 37% in LAD between cycles. The LAI_g integration over the sugarcane main development phases (formative, grand growth and maturity) resulted in 14%, 46% and 40% of total LAD in the first cycle, respectively; while in the second cycle the percentages were 6%, 39% and 55%, respectively. Hence in the second cycle

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most of the total LAD (55%) covered the last growing phase, which is characterized by the slowdown of physiological processes (van Heerden et al., 2010). There was an apparent decrease in the green leaf biomass sampled on 31/01/2006 but the difference was not significant (p=0.001); the same is true with respect to stalks between the last two sampling dates in the first cycle. The decreases in LAI_g observed over the final 50 days were promoted by the aerial spraying of chemical ripeners; these produce a rapid decline in stalk elongation, lower rates of leaf development and thus more sucrose being available for storage in stalks (Lopes et al., 2011).

3.5-Seasonal patterns of NEE, GEP and R_E

The time series of daily totals of GEP and $R_{\rm E}$, as well the cumulative NEE, are depicted in Fig. 3. The GEP in the first cycle shows a steady increase from the harvest in April 2005 until 10/01/2006, values above 15 g C m⁻² day⁻¹ were then achieved during the main growth period from December to February of 2006, after which they slowly decreased – a characteristic of the maturity phase. The beginning of the second cycle was marked by a slower re-growth from May to October of 2006, as the GEP increase was enhanced only after the onset of the rainy season in October (Fig. 1b). The fully developed canopy conditions were observed in February of 2007, when the GEP reached about the same value as previously. The seasonal patterns of daily totals of ecosystem respiration exhibited a dependency on GEP (see Migliavacca et al., 2011; Suleau et al., 2011). Peak GEP values obtained in both cycles (~21 g C m⁻² day⁻¹) were similar to the values found for maize (Béziat et al., 2009; Stella et al., 2009; Kalfas et al., 2011), as was the observed range of $R_{\rm E}$ from 1 to 10 g C m⁻² day⁻¹.

Based on the change of signal from positive to negative in the cumulative NEE (Fig. 3), which was observed 109 days after the harvest (30/07/2005, day 211) in the first cycle but 214 days after the harvest (11/12/2006, day 345) in the second cycle, the rate of bud emergence from stubble during the second cycle probably exhibited some effect of the early-season water deficit. All available soil moisture (Fig. 1b) was extracted during the initial 120 days of growth (136 mm m⁻¹; see Cabral et al., 2012) and the associated decrease in the water potential could have caused not only lower rates of germination but also a more irregular time distribution of the germinating buds (Moreira and Cardoso, 1998; Inman-Bamber and Smith, 2005). Such water stress can have important implications for crop yield at final harvest (see Robertson et al., 1999), as can be seen here in Fig. 2.

<< Place Figure 3 about here>>

The monthly totals of gross ecosystem productivity (GEP), ecosystem respiration (R_E) and their ratios (GEP/ R_E) are presented in Fig. 4. The initial two months of the first cycle exhibited R_E totals greater than GEP, but in June of 2005 the assimilation exceeded the respiration; however, the GEP/ R_E ratios were close to, or above, two for most of the cycle. These GEP/ R_E ratios around two imply that NEP~ R_E and indicate that the autotrophic respiration drives the carbon fluxes, although when GEP substantially exceeds R_E the system withdrawal of available nutrients is maximized (Falge et al., 2002). These results confirm the importance of the biomass in the Q_{10} sensitivity as discussed in Section 3.1, because in crops the large temperature sensitivities mainly result from leaf assimilation and plant growth rather than being a direct R_H response to temperature (Aubinet et al., 2009).

Contrasting with the first cycle, the sugarcane agrosystem was a source of CO₂ to the atmosphere during the initial five-month period of re-growth in the second cycle (GEP/R_E<1). This resulted from the sugarcane plants being smaller due to receiving less rainfall (Fig. 1b) and consequently having lower rates of photosynthesis (GEP) and R_E (van der Molen et al., 2011). Notice that GEP exceeded R_E only in November of 2006. From the totals summarized in Table 2, the observed decreases in NEE and GEP between cycles were 51% and 25%, respectively. Further, during the initial 150 days (tillering phase) in the second cycle the sums of GEP and R_E were lower than in the first cycle by 62% and 20%, respectively, in agreement with the results of Schwalm et al. (2010), which indicated that GEP sensitivity to water stress was approximately 50% greater than R_E with the agricultural areas exhibiting the highest sensitivity. The respiratory costs (R_E/GEP) were considerably higher in the beginning of the re-growth particularly in the second cycle, and decreased toward the final phase (see Fig. 4). The total costs in each cycle were 46% and 65%, respectively, indicating that in the second cycle the respiration costs were 41% greater (Table 2). The R_E/GEP observed in the first sugarcane cycle was lower than the ratios reported for maize, soybean and wheat crops, i.e., from 0.55–0.66 (Law et al., 2002; Suyker et al., 2005; Verma et al., 2005; Moureaux et al., 2008), although the upper limit of the interval was achieved in the second cycle.

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480 <<Insert Table 2 about here>>

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3.6 – Water use efficiency (WUE)

Accordingly to Suyker and Verma (2010), the consequence of an extended dry period in maize is reduction in GEP, as well as in the evapotranspiration (ET). The sugarcane evapotranspiration reduction between cycles, reported by Cabral et al. (2012), was of the same order (17%) as found here for the GEP decrease. Based on the same evapotranspiration data (Cabral et al., 2012), we calculated the water use efficiency (WUE) defined as the GEP/ET ratio (Suyker and Verma, 2010) over the three sugarcane main phases (Table 2). The sugarcane water use efficiency was lower in the second cycle during tillering (17%), grand growth (16%) and the decrease in the final phase (maturation) represented the same (5%) as the overall WUE decrease between cycles (totals in Table 2). However, the low WUE values obtained in the initial sugarcane phase are comparable to the maximum WUE for miscanthus (2.2 g C kg⁻¹ H₂O) reported by VanLoocke et al. (2012) and soybean (Suyker and Verma, 2010); and the WUEs in the second phase were similar to the average growing season totals for maize (3.2 g C kg⁻¹ H₂O) given by Suyker and Verma (2010).

3.7 - NPP and R_E partition

The harvested totals of biomass and the estimated net primary productivities (NPP) are summarized in Table 3. The partition of R_E into heterotrophic (R_H) and autotrophic (R_A) respiration was obtained from the totals in Tables 2 and 3, as the residuals (R_A =GEP-NPP; R_H = R_E - R_A) and the error propagation was calculated using the error accumulation principle (Aurela et al., 2002; Béziat et al., 2009). The first cycle R_H and R_A totals were

249±132 and 1399±131 g C m⁻², respectively and in the second cycle 422±112 and 508 1337±99 g C m⁻², respectively. R_H increased 69% (173 g C m⁻²) in the second cycle, but 509 510 R_A decreased 4% (62 g C m⁻²) while R_E increased 7% (111 g C m⁻²) between cycles. 511 During the initial 150 days in the second cycle (Table 2) the cumulative NEE represented a loss of 105±12 g C m⁻² and the likely sources were the old root system 512 513 turnover estimated as 45±15 g C m⁻² (see below) and part of the biomass remaining on the soil due to incomplete burning, an estimate of this (81±73 g C m⁻²) was obtained by 514 515 assuming an average decay of 51% based on early harvest data in Australia (see Galdos 516 et al., 2010). Therefore the carbon emission from this heterotrophic short-term supply of labile organic carbon (van der Molen et al., 2011) resulted in 126±74 g C m⁻², which did 517 518 not differ from the cumulative NEE. 519 The R_H represented 15% and 24% of R_E in each cycle respectively, while R_A achieved 520 85% and 76%, overall the R_H and R_A components of respiration accounted for 20% and 521 80% of R_E. This result confirms that R_A is the major component of respiration (Falge et 522 al., 2002) and represents from 60% to 80% of R_E (Moureaux et al., 2008; Aubinet et al, 523 2009; van der Molen et al., 2011; Suleau et al., 2011).

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3.8 – Carbon use efficiency (CUE)

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The carbon use efficiency (CUE=NPP/GEP) varied from 0.61 in the first cycle to 0.51 in the second, implying that approximately 20% less carbon was assimilated in the second cycle. The observed R_A/GEP ratios represented 0.39 and 0.49 in each cycle respectively, agreeing with the empirical evidence (Van Oijen et al., 2010) that the

 R_A/GEP ratio is constrained to a narrow range (0.4–0.5). Thus, the autotrophic respiration utilized 26% more resources in the second sugarcane cycle. The parallel reductions in GEP and R_E (62% and 20%) were observed only during the initial phase of the second cycle (see Table 2), when the agrosystem was a source of CO_2 to the atmosphere (105±12 g C m⁻²). For the other phases although GEP was lower than during the first cycle, R_E increased. The observed decreases in NPP and NEP occurred jointly as the GEP decreased between cycles (-893 g C m⁻²), but was not followed by R_E which in fact increased (111 g C m⁻²). NEP was therefore dominated by NPP (see Reichstein et al. 2007).

3.9 – Net ecosystem carbon balance (NECB)

In this study the net ecosystem carbon balance (NECB) is defined as the sum of NEE and the carbon losses during the harvest by non-respiratory processes (Anthoni et al., 2004), which in this sugarcane plantation should include the biomass burning and the stalk removal (seeds and fertilizers inputs not considered here). The total dead leaf biomass and a fraction of the green leaves (37%) were burnt (see Cerri et al., 2011), resulting in direct carbon emissions of 630±31 and 406±23 g C m⁻² in the first and second cycle, respectively. Accounting for the stalk removal (1436±118 and 957±49 g C m⁻², in each cycle respectively) the NECB of the sugarcane agrosystem was approximately carbon neutral (102±130 g C m⁻²) in the first cycle due to the large uncertainty, and a carbon source (403±83 g C m⁻²) in the second cycle, as a consequence of the management practices as well the water shortage observed at the beginning of the second cycle.

The NECB represents the changes in non-harvestable carbon pools as the soil organic carbon, root biomass and aboveground residue (Anthoni et al., 2004; Verma et al., 2005; Zeri et al., 2011). The amount of sugarcane residue remaining aboveground after the first cycle harvest, due to the incomplete burning was 159±73 g C m⁻² and the estimated root biomass turnover was 45±15 g C m⁻², therefore the sum of these heterotrophic respiration sources represented 204±74 g C m⁻², which subtracted from NECB resulted in 199±112 g C m⁻² originating from the soil pool. This estimate assumes that 100% of the aboveground residues had decomposed at the end of the second cycle, which is of course an overestimation (see Robertson and Thorburn, 2007). There are also great uncertainties associated with the estimated root system. The results of root biomass reported here were based on samples taken to a maximum depth of 1.0 m. Battie Laclau and Laclau (2009) indicated that 50% of the root intersects (see Chopart et al., 2008) were below 1 m depth (see Cabral et al., 2012); therefore the average ratio of 0.122 is probably an underestimate of the real root-to-shoot ratio as well as the root turnover figure (17%) given by Ball-Coelho et al. (1992). Denmead et al. (2010b), working in Australia, estimated that the sugarcane root biomass represented 32% of the harvested biomass, if we suppose this figure represents the sugarcane in Brazil as well, and applying it to the first cycle aboveground net primary productivity (ANPP in Table 3) the root biomass and turnover would decrease the loss of carbon originating from the soil by approximately 50%. The integrated soil organic carbon obtained after the harvest in 2007 was 2880±340 g C m⁻² in the first 0.3 m of soil and $6350\pm500~g~C~m^{-2}$ down to 1 m depth. Therefore the estimated emissions of 200 g C m⁻² would represent 7% to 3% of the soil carbon stock depending on the soil layer thickness. Temporal changes in soil carbon are small and difficult to detect by soil sampling in the short term (Verma et al., 2005) as they exhibit large coefficients of

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583	variation. Changes in soil carbon of approximately 100 g C m ⁻² yr ⁻¹ requires time
584	periods greater than 10 years to be observed with confidence (Denmead et al., 2010a,b)
585	However, a change in farming practice from burning to trash conservation would
586	benefit cane productivity and increase soil carbon stocks (Resende et al., 2006; Galdos
587	et al., 2009; Pinheiro et al., 2010; Cerri et al., 2011).
588	We compared the carbon balance in this study to several others. In some agricultural
589	systems, carbon balance was reported to be near neutral but with considerable
590	uncertainty from 13±33 to 138±239 g C m ⁻² yr ⁻¹ (Robertson et al, 2000; Ceschia et al.,
591	2010; Ciais et al., 2010). However other crop studies report carbon losses of 95±87 g C
592	m ⁻² yr ⁻¹ , e.g., Kutsch et al. (2010), based on seven sites; Béziat et al. (2009) who found
593	one site neutral and another a carbon source (100 g C m ⁻² yr ⁻¹) depending on the
594	management, while Alberti et al. (2010) estimated greater losses in alfalfa (354 g C m ⁻²
595	yr ⁻¹) than in maize (96 g C m ⁻² yr ⁻¹), although for winter-wheat season Moureaux et al
596	(2008) found a sink of -630 g C m ⁻² . Verma et al. (2005) also concluded that the
597	irrigated maize-soybean rotation was a moderate source of carbon (70 to 102 g C m ⁻²
598	yr ⁻¹) while under rainfed conditions Zeri et al. (2011) found a source of approximately
599	200 g C m ⁻² yr ⁻¹ . According to Anderson-Teixeira et al. (2009) significant soil carbon
600	losses resulted from land-use change to biofuel agriculture, particularly when natural
601	vegetation was converted to sugarcane agriculture.
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603	3.10 - Environmental controls

3.10 - Environmental controls

3.10.1 - GEP and $R_{\rm E}$ dependency on $LAI_{\rm g}$

The scatter plot between daily means of half-hour GEP averaged when PAR was above 1500 μmol quanta m⁻² s⁻¹ versus LAI_g (Fig. 5a) indicates that LAI_g accounted for more than 90% of the GEP variability at light saturation in each cycle, in agreement with other crops and grassland studies where LAI_g usually explains above than 80% of the GEP variance (Flanagan et al., 2002; Xu et al., 2004; Aires et al., 2008; Suyker and Verma, 2010; Polley et al., 2011). The average GEP increases were 9.9 and 11.7 μmol $CO_2 \, m^{-2}$ of leaf s^{-1} in each cycle, respectively per unit of LAI_g increase, therefore the slope of the GEP and LAI_g relationship was 18% greater in the second cycle, despite the lower yield (see Table 3). Roberts et al. (1990) have already noticed this effect in sugarcane as the compensatory growth after re-watering, because photosynthesis is less sensitive to soil water limitations than the crop growth (Polley et al., 2011). Although the recovery is partial (Ghannoum, 2009), it may contribute to the attenuation of the initial water stress effects on the final yield depending on the climate conditions towards the end of the cycle and if it is extended, with the application of ripeners and harvest postponed, as for example observed by Cabral et al. (2003) who reported similar sugar cane yields (107 t ha⁻¹) in a more productive area, at the end of the fourth and sixth ratoons, mainly because the latter cycle was 40 days longer.

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The daily totals of R_E versus 1<LAI $_g$ <5 interval, obtained by the polynomial interpolation of the data in Fig. 2, for both cycles are presented in Fig. 5b. LAI $_g$ also explained 69% and 56% of the R_E variability in each cycle respectively, indicating that the elevated apparent Q_{10} obtained here is likely to be a consequence of the positive covariation between larger LAI $_g$ (see Suyker et al., 2004; Aubinet et al., 2009) and

summer temperatures mediated by the soil moisture content (Davidson et al., 2006) whose lower values observed during the initial stages of re-growth reduced the total respiration from biomass and soil. Notice that R_E was on average approximately 3.5 g C m^{-2} day⁻¹ greater in the second cycle for the same LAI_g (Fig. 5b), therefore precluding the estimation of R_E based in LAI_g intervals as reported for other agrosystems (Suyker et al, 2004).

3.10.2 - GEP versus PAR and VPD

The relationships between half-hour ensembles of GEP and PAR, calculated over LAIg intervals are depicted in Fig. 6 for both cycles, as well as the fitted rectangular hyperbolas (GEP = α GEP_{max} PAR/(GEP_{max}+ α PAR)). The curves were similar for 1<LAIg<2 (Fig. 6a) and probably exhibit the effect of incomplete absorption of the light by the plants as the canopy was not fully-closed (Valentini et al., 1995). However contrary to expectation, the initial slope of the light curve which represents the apparent photon yield (α) and the asymptotic value (GEP_{max}) in the 2<LAIg<3 and 3<LAIg<4 intervals (Fig. 6b) were greater in the second cycle, although when nearly complete canopy cover was achieved (see Shuttleworth and Gurney, 1990). For the second cycle, in the 3<LAIg<4 the light curve (Fig. 6c) exhibited afternoon depression (Hirasawa and Hsiao, 1999; Xu et al., 2009; Lasslop et al., 2010), whilst no signs of saturation were observed in the first cycle.

Notwithstanding, the α range found, i.e., from 0.03 to 0.07 mol CO₂ mol⁻¹ quanta, was similar to the values obtained for maize (Suyker et al., 2004 and 2005; Béziat et al., 2009; Arkebauer et al., 2009); as well as the GEP_{max} (from 8 to 53 µmol CO₂ m⁻² s⁻¹) for

similar LAI_g achieved in the sugarcane ratoons when compared with maize and besides the fact that the assimilation rates in older sugarcane plants are lower than in new plants (Allison et al., 1997; Vu et al., 2006).

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The growth cycles occurred at different times of the years (see Fig. 2) as noticed from the higher PAR (Fig. 6a) over the interval $1 < LAI_g < 2$ in the second cycle because it covered the period October–November of 2006 (days 291-328) while in the first cycle it was observed in July–August of 2005 (days 186-233), hence for the other LAI_g intervals depicted in Fig. 6 (b-c) the first cycle also occurred earlier in the year. The GEP and PAR relationships therefore probably include the effects of other driving variables. We calculated the half-hourly averages of GEP normalized by PAR bins (see Stella et al., 2009); these were then plotted versus saturation deficits (VPD), covering the same intervals of LAI_g already used. These results are depicted in Fig. 7.

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The sugarcane experienced higher humidity deficits in the first cycle for $LAI_g>2$ (Fig. 7b), somewhat explaining the observed patterns in the light curves for $LAI_g<2$ (Fig. 6 b,c). However the GPP/PAR ratios were relatively less sensitive to VPD in the first cycle as the ratios in the second cycle exhibited a sharp decrease with VPD, despite the smaller range. Therefore, the GEP decreases observed in the sugarcane mainly resulted from the high atmospheric demand for water exceeding soil-plant hydraulic system (see Lizaso et al., 2005) and the consequent stomatal limitation as found by Cabral et al.

(2012). These authors have previously reported an exponential decrease in stomatal conductance with VPD for this sugarcane plantation. According to Fletcher et al. (2007) decreases in the radiation use efficiency with increasing VPD found in corn and sorghum were consistent with the afternoon depression of photosynthesis. Notice that in the first cycle LAI_g=4 was achieved at 211 days after harvest (9/09/2005, day 313) against 275 days in the second cycle (10/02/2007; day 41), during the maturation phase characterized by lower physiological rates (Ramesh, 2000; Tejera et al., 2007; van Heerden et al, 2010). The extrapolation of the fitted curves (Fig. 7b, c) representing the second sugarcane cycle (gray lines) towards the deficits experienced in the first cycle would produce average decreases in the GEP/PAR ratios of approximately 50%. Thus physiological effects on transpiration, photosynthesis and respiration (Zhao et al., 2010) are likely to become more common as sugarcane is increasingly grown on sandy soils, where yields tend to be more vulnerable to environmental stresses such as nutrient and water deficits (Ezenwa et al., 2005; Silva et al., 2007).

3.10.3 - GEP and SLN

In order to assess whether the changes in GEP between sugarcane cycles might also be related to other causes, unrelated to the stomatal conductance, we present the time series of specific leaf nitrogen (SLN) expressed on a green leaf area basis, during both ratoons (Fig. 8 a) and the mean ratios GEP/LAI $_g$ for PAR>1000 μ mol quanta m $^{-2}$ s $^{-1}$ (Fig. 8 b), which are related to the amount of photosynthetic material per unit leaf area, averaged over seven days, centered on the days of the SLN determinations.

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The overall range of SLN, from 0.7±0.03 to 1.7±0.13 g N m⁻² of leaf is within the 708 709 interval reported for C4 grasses and sugarcane (Ghannoum et al., 2005; Park et al., 710 2005) and the decrease in SLN with crop age has already been found in different types 711 of plants (Wood et al., 1996; Allison et al., 1997; Flanagan et al., 2002; Turner et al., 712 2003; Wang et al., 2012). The associated decreases in the mean GEP/LAI_g show the 713 dependence of the photosynthetic response to irradiance on the leaf N content (Gastal and Lemaire, 2002; McCormick et al., 2008). The values around 15 μ mol CO₂ m⁻² of 714 leaf s⁻¹, which represent the canopy integrated GEP, are comparable to the whole plant 715 716 photosynthesis observed by Inman-Bamber et al. (2008), who noticed that this figure is 717 approximately half the photosynthetic rate of young leaves exposed to the same 718 radiation. 719 The differences in average SLN between cycles calculated over the three main phases of 720 the sugarcane cycle (Ramesh, 2000) were not significant (t Test, p=0.05) in agreement 721 with the results of Park et al. (2005), who compared plant and ratoon crops. 722 The same comparison of GEP/LAI_g mean ratios between cycles showed that only 723 during the second growing phase (grand growth: 150-240 DAH) was the difference 724 between cycles significant. However, in the second cycle, a secondary peak in SLN was 725 observed between 150 and 200 days after harvest (day 290-332/2006) (Fig. 8a); this was 726 due to the onset of rainfall (188 mm, see Fig. 1b) promoting a flush of new leaves with 727 high N content and consequently higher assimilation rates (McCormick et al., 2008). 728 Photosynthesis under more severe conditions may be more sensitive to stress because 729 the decrease in transpiration will induce an increase in leaf temperature (Lopes et al., 730 2011). The viability of sugarcane ratooning is determined by the condition of the old 731 root system during the mainly dry period after harvest. Its condition is critical to the

survival of the stool and growth of the following ratoon crop (Smith et al., 2005).

Results from sugarcane model simulations on adaptation to drought (see Inman Bamber et al., 2012) indicate that increased rooting depth results in an up to 21% increase in mean dry biomass yield depending on the climate and soils, but the spraying of chemical ripeners (Inman-Bamber et al., 2008; Lopes et al., 2011), which is a common pre-harvest practice in sugarcane, can also adversely impact the ratooning, as the early induced maturation reduces the level of photo-assimilates partitioned into the root system (Viator et al., 2010).

4-Conclusions

To meet the growing demand for biofuel, sugarcane plantations in Brazil are replacing existing pastures and expanding into areas with a strong inter-annual variability of climate. This trend is expected to continue. Although limited to two cycles our results showed that the crop yields are likely to be more variable, particularly in areas of sandy soils where yields can be more vulnerable to environmental stresses such as nutrient and water deficits promoting physiological impacts on photosynthesis and respiration. However as observed here, in the first cycle, the decrease in the radiation use efficiency could sustain crop yields in a water-limited environment.

Our results also show that the NECB measured above the sugarcane plantation was approximately carbon neutral (102±130 g C m⁻² yr⁻¹) in a normal cycle (second ratoon), whose yield attained the expected regional average. However, it was a carbon source (403±83 g C m⁻² yr⁻¹) in the following cycle (third ratoon) when the yield was 19% lower than the regional average and 26% lower than the previous ratoon. The carbon

losses resulted mainly from harvest but also the reduced re-growth resulting from the lower rainfall during the initial 150 days of the second cycle, when the ecosystem respiration (mainly heterotrophic) was greater than the assimilation due to the prolonged period with partially covered soil. Although the plantation re-established the growth after the onset of the summer rainfall, the assimilated CO₂ did not offset the net carbon emissions associated with the stubble burning and stalk removal. For the conditions observed here, our results indicate that the avoidance of the carbon released during burning would lead to a negative (sink) net ecosystem carbon balance in the first cycle and a neutral balance in the second cycle. In approximately 40% of the total area of sugarcane in Brazil, pre-harvest burning is not used and the trash remains on the soil surface, although most of the trash decomposes during the cycle it is generally assumed that a change in farming practice from burning to trash conservation will have benefits for cane productivity and increase soil carbon stocks. Our results stress the importance of implementing a biomass burning ban, which must be pursued by the Brazilian government and industry.

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Figures captions Figure 1- Time series of 30-minute averages/totals of (a) air temperature (black lines) and water vapor saturation deficit (VPD, gray dashed lines); (b) rainfall (black lines) and soil water content in the first meter of soil (gray line); (c) photosynthetically active radiation (PAR, black area) and friction velocity (U_{*}, gray area). Figure 2- Aboveground biomass: green leaves (circles); dead leaves (squares) and stalks (triangles). The green leaf area index (LAI_p) is represented by the gray line. The error bars represent \pm standard deviation. Figure 3 – Daily totals (g C m⁻² day⁻¹) of gross ecosystem productivity (GEP, thick black line), ecosystem respiration (R_F, gray line) and the cumulative net ecosystem exchange (NEE, thin black line) above the sugarcane plantation. The horizontal gray arrows indicate the main phases duration of the cycles (see text) and the black bar the time of the first cycle harvest (11/05/2006, day 131). Figure 4 - Monthly totals of gross ecosystem productivity (GEP, gray bars), ecosystem

Figure 4 - Monthly totals of gross ecosystem productivity (GEP, gray bars), ecosystem respiration (R_E, black bars) and their ratios (GEP/R_E, transparent bars) observed during the two cycles of sugarcane.

Figure 5 – Scatter plots of (a) daily mean gross ecosystem productivity (GEP, µmol CO₂ m⁻² s⁻¹) averaged when PAR>1500 µmol quanta m⁻² s⁻¹ and (b) daily totals of ecosystem respiration (R_E, g C m⁻² day⁻¹) versus green leaf area index (LAI_g, m² m⁻²) during the first (black circles) and second (gray squares) sugarcane cycles.

Figure 6 – Scatter plots of half-hourly ensembles of gross ecosystem productivity (GEP, µmol CO₂ m⁻² s⁻¹) versus photosynthetic active radiation (PAR, µmol quanta m⁻² s⁻¹) calculated over green leaf area index intervals: (a) 1<LAI_g<2; (b) 2<LAI_g<3; (c) 3<LAI_g<4. The lines represent the fitted hyperbolas in the first (black circles and lines) and second (gray squares and lines) sugar cane cycles. The error bars represent the

and second (gray squares and lines) sugar cane cycles. The error bars represent the ±standard deviations.

Figure 7 – Scatter plots of gross ecosystem productivity (GEP) normalized by the photosynthetic active radiation (PAR) bins versus water vapor saturation deficits (VPD, kPa) calculated over green leaf area index intervals: (a) 1<LAI_g<2; (b) 2<LAI_g<3; (c) 3<LAI_g<4, during the first (black circles) and second (gray squares) sugarcane cycles. The bars represent +standard deviation due to the ordinate log scale.

Figure 8 –Time series of (a) green leaves specific leaf nitrogen (SLN) and (b) mean GEP/LAI_g ratios calculated for PAR>1000 μ mol quanta m⁻² s⁻¹ during the first (black circles) and second (gray squares) sugarcane cycles plotted as a function of the days after harvests (day 103/2005 and 132/2006, respectively). The error bars represent the \pm standard deviations.

1393 standard dev

Table 1 – Exponential relationships between night NEE (= R_E) and T_{air} (R_E = a exp^{bTair}) for different intervals of soil water content (SWC, mm) in the 0-0.9 m layer. The coefficients are followed by the standard errors (\pm SE). The standard error of the estimate is represented by \pm SEE and the number of degrees of freedom by DF. The significance is indicated by: ${}^cP(>|t|) < 0.001$ and ${}^dP(>|t|) < 0.05$. Q_{10} (=exp^{10b}) is the factor by which the R_E is multiplied when T_{air} increases by $10^{\circ}C$.

SWC intervals	a	b	± SSE (μmol CO ₂ m ⁻² s ⁻¹)	R^2	Q ₁₀	DF
(mm)						
(11111)						
< 100	1.0097±0.082 ^d	0.0196±0.003°	1.054	0.66	1.22	91
100 -120	1.0532±0.048 ^d	0.0434±0.002 °	1.058	0.88	1.54	157
120-140	1.0368±0.033	0.0636±0.002 °	1.058	0.91	1.89	256
140-160	1.0593±0.054	0.0725±0.003 °	1.062	0.87	2.06	249
>160	1.0390±0.049	0.0882±0.002 °	1.057	0.90	2.42	86

Table 2 - Cumulative fluxes and associated uncertainties (g C m⁻²) observed during the two cycles of sugarcane plantation calculated over the three main phases as indicated by the number of days after harvest (DAH) intervals. The water use efficiency (WUE=GEP/ET) was calculated based on the evapotranspiration data (mm day⁻¹) from Cabral et al. (2012).

Cycles (DAH)	NEE	GEP	$R_{\rm E}$	WUE	
	g C m ⁻²			g C kg ⁻¹ H ₂ O	
Cycle 1					
1-150 ¹	-175±8	585±7	410±4	2.9	
151-240 ²	-639±15	987±15	348±4	4.3	
241-392 ³	-1150±25	2040±24	890±7	5.0	
Total	-1964±44	3612±46	1648±14	4.3	
Cycle 2					
1-150 ¹	105±12	225±7	329±10	2.4	
151-240 ²	-290±36	846±31	555±19	3.6	
241-373 ³	-775±51	1649±45	874±24	4.8	
Total	-960±64	2719±83	1759±53	4.1	

Sugarcane phases: ¹Tillering, ²Grand Growth and ³Maturation.

Table 3 – Components of sugarcane dry biomass (g C m^{-2}) measured and estimated at the harvests. The \pm represents the standard error of the mean (10 samples).

Harvests Dates	103/2005	131/2006	140/2007		
		Cycle 1	Cycle2		
Biomass Components	g C m ⁻²				
Green Leaves	214±12	157±9	123±6		
D. 111	405 - 24	572 - 20	260.22		
Dead Leaves ¹	405±24	572±30	360±22		
Stalks	1518±82	1436±118	957±49		
Aboveground NPP	2137±86	2165±122	1440±54		
Roots	261±10	264±15	176±7		
NPP ²		2213±123	1382±54		

We suppose the decay in the dead leaves was negligible.

² NPP is the sum of aboveground NPP (ANPP) and the difference of roots biomass between harvests and includes the estimate of root turnover after the harvests (17%).





















