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Contact CEH NORA team at
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1 Fluxes of CO₂ above a sugarcane plantation in Brazil

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4 Osvaldo M. R. Cabral^{1*}, Humberto R. Rocha², John H. Gash³, Marcos A. V. Ligo¹,

5 Nilza Patrícia Ramos¹, Ana Paula Packer¹, Eunice Reis Batista¹

6 ¹Embrapa Meio Ambiente, CEP 13820-000, Jaguariúna-SP, Brazil

7 ²Universidade de São Paulo, R. do Matão 1226 CEP 05508-090, São Paulo, SP, Brazil

8 ³Centre for Ecology and Hydrology, Wallingford OX10 8BB, United Kingdom

9

10

11

12

13 *Corresponding Author:

14 Osvaldo M. R. Cabral

15 Embrapa Meio Ambiente, Jaguariúna-SP, Brazil

16 CEP 13820-000

17

18 osvaldo.cabral@embrapa.br

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27 **Abstract**

28

29 Fluxes of CO₂ 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 m⁻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_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±130 g C m⁻² and 403±84 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 m⁻²). 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 H₂O) decreased only 5% in the second cycle.

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59 Key words: CO₂ fluxes; sugarcane; respiration; eddy covariance; biomass; soil water
60 deficits

61 **1-Introduction**

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63 The increasing global demand for sustainable energy sources has led to more
64 agricultural land being used for biofuel production. There are many concerns about the
65 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
67 the carbon cycle (Verma et al., 2005; Beringer et al., 2011; Zenone et al., 2011). The
68 greenhouse gas (GHG) balance of an energy crop will depend on the crop type, climate,
69 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.,
71 2011). The same is true when residues from agriculture and forestry are used as biofuel.
72 Although it is often assumed that the carbon input and output of croplands are in
73 equilibrium (Moors et al., 2010), this crude simplification of the production cycle can
74 produce misleading results (see Moureaux et al., 2008; Aubinet et al., 2009; Béziat et
75 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 pre-
77 harvest burning is practiced in approximately 60% of the total area (Pinheiro et al.,
78 2010). After the first year of establishment the yields of the re-growth from the stubble
79 (ratoon crop) decrease at an average rate of approximately 10% per year (Cabral et al.,
80 2012). After four successive harvests the yield falls to 7.0 kg m^{-2} and the plantation is
81 re-established (Macedo et al., 2008). In 2009, Brazil produced 696 Tg of sugarcane,
82 from an area of $86,800 \text{ km}^2$, with 54% of this being in the southeastern state of São
83 Paulo (Brasil, 2010). According to the expected domestic and international demands,
84 over the next 10 years sugar and bioethanol production should increase by 49% and
85 127%, respectively. Over the same period the planted area is expected to increase by

86 49% (Brasil, 2010), i.e., to some 129,000 km². These figures imply that the area
87 available for expansion is not limiting; and indeed it has been estimated that the total
88 area available is 647,000 km², with approximately 30% classified as highly productive
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_E) 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

111 by non-respiratory processes such as run-off, pre-harvest burning and harvest (Anthoni
112 et al., 2004; Verma et al., 2005), as well as the carbon inputs as fertilizer and seeds
113 (Smith et al, 2010).
114 The objectives of this work were to observe the seasonal dynamics in the net ecosystem
115 exchange in a representative sugarcane agrosystem and to characterize how its
116 constituents, GEP and R_E , respond to environmental factors. The establishment of the
117 net ecosystem carbon balance (NECB) and the water use efficiency (WUE) of the
118 sugarcane crop, will contribute to reduction of the large uncertainty in the carbon
119 balance of tropical C4 ecosystems.

120

121 **2-Methods**

122

123 **2.1-Site**

124

125 The sugarcane (*Saccharum* spp hybrids) plantation is located in Luiz Antonio
126 municipality in São Paulo State, Brazil (21° 38' S, 47° 47' W) at 552 m altitude.
127 The maximum rain falls in December (274±97 mm month⁻¹) and the minimum in July
128 and August (27±34 mm month⁻¹); the mean annual precipitation (for years 1971 to
129 2007) and its standard deviation are 1517±274 mm. The mean temperatures in January
130 and July are 24 °C and 19 °C, respectively, and the annual mean is 22 °C. The mean dry
131 bulk density of the sandy soil (Typic Haplustox) down to 2.6 m depth is 1500 kg m⁻³,
132 and the available soil water in the first meter is 136 mm. The area (> 400 ha) exhibited a
133 small slope of less than 2%. During unstable conditions, flux footprint calculations (see
134 Hsieh et al., 2000) indicated that >90% of the measured fluxes emanated from the
135 uniform crop of sugarcane under study. The distance between planting rows was 1.4 m

136 and the maximum canopy height achieved was approximately 5 m (further details are
137 given by Cabral et al., 2012; Cuadra et al., 2012).
138 The measurements reported here began just after the second harvest (first ratoon), which
139 occurred in 13/04/2005 (day 103) and extended to the harvest in 20/05/2007 (day 140)
140 covering two cycles which represented the second (ended in 11/05/2006, day 131) and
141 third re-growth from the stubble (ratoons). The pre-harvest practices included the
142 burning and the aerial spraying of chemical ripeners to increase the sucrose
143 accumulation in the sugarcane stalks (see Inman-Bamber et al., 2008; Dalley and
144 Richard, 2010); spraying occurred in 22/03/2006 and 01/04/2007, respectively. One
145 week after both harvests the area received 400 kg ha⁻¹ of limestone (CaCO₃), which was
146 equivalent to 4.8 g C m⁻² supposing it was in the form of calcite; and urea-based
147 fertilizer representing 56 kg N ha⁻¹ and 150 kg K₂O ha⁻¹ of potassium. The planted
148 varieties (SP81-3250, SP83-2847 and RB86-7515), the soil and achieved yields were
149 representative of southern Brazilian conditions (see Marin et al., 2011; Cabral et al.,
150 2012).

151

152 **2.2-Instrumentation**

153

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

161 performed manually every two weeks. A datalogger (CR1000, Campbell SI, Logan, UT,
162 USA) recorded all the raw data (21 Hz) for post-processing.
163 The air temperature, humidity (HMP45, Vaisala, Helsinki, Finland) and rainfall (TB4,
164 Hydrological Services Pty. Ltd., NSW, Australia) were measured at 6 m height; the net
165 radiation (R_n), global radiation (R_g) and photosynthetically active radiation (PAR)
166 sensors (Kipp and Zonen, Delft, The Netherlands) were fixed at 7 m height on a
167 horizontal boom 2.5 m away from the tower. The soil moisture content (SWC) was
168 measured by 10 reflectometers (CS615, Campbell SI, Logan, UT, USA) installed
169 vertically (0.3 m layers), down to 3 m depth. The soil heat flux was obtained from the
170 mean of four plates (REBS, Seattle, WA, USA) installed within and between the
171 planting rows, buried at 25 mm depth.

172

173 **2.3-Data processing**

174

175 CO_2 flux (F_{CO_2}) was obtained from the covariances between the fluctuations (with
176 respect to 30-minute block averages) of vertical wind speed (w) and CO_2 air
177 concentration. The effects of sonic anemometer misalignment or topographically
178 induced flow were accounted for with a double rotation forcing the mean vertical
179 windspeed to zero (Kaimal and Finnigan, 1994); the time lag between w and CO_2 was
180 removed prior to the calculation of the covariances; this procedure also corrects for the
181 sensor separation and phase-shift (see Ibrom et al., 2007). The half-hour averages were
182 also discarded if the values were outside predefined realistic bounds (see Zeri et al.,
183 2011) and filtered to remove periods of non-stationarity (Foken et al., 2004). During
184 stable atmospheric conditions the friction velocity (U_*) filter was applied (Aubinet et
185 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
190 ($[1+(2\pi f\tau_s)^2]^{-1}$, where f is the natural frequency) is applied to the measured cospectra of
191 heat fluxes in order to represent the observed degraded spectra of the F_{CO_2} fluxes and
192 obtain the necessary correction factor (see Aubinet et al., 2000).

193 The CO_2 storage (S_{CO_2}) in the air column below the eddy-covariance system necessary
194 to calculate the net ecosystem exchange ($NEE = F_{CO_2} + S_{CO_2}$) was estimated from the
195 time variation of air CO_2 density measured at 9 m height by the IRGA, with the implicit
196 assumption that the CO_2 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 CO_2 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).

211 The gross ecosystem productivity (GEP) was calculated as the difference between the
212 ecosystem respiration (R_E) during daylight hours, obtained from the fitted exponential
213 relationships between nighttime CO_2 fluxes (R_E) and air temperature (see Reichstein et
214 al., 2002) and the observed NEE ($=R_E-GEP$), whose negative flux indicated a net
215 downward transport of CO_2 .

216

217 The uncertainties associated with the eddy-covariance fluxes (see Moncrieff et al.,
218 1996; Goulden et al., 1996) covered the errors due to the gap-filling models (Falge et
219 al., 2001; Oren et al. 2006), the random errors (Hollinger and Richardson, 2005) and the
220 selective systematic bias introduced by the NEE estimates under low turbulence
221 conditions (Aurela et al., 2002; Papale et al., 2006; Béziat et al., 2009).

222

223 The quality of CO_2 fluxes was assured by the energy balance closure (see Wilson et al.,
224 2001; Culf et al., 2004; Leuning et al., 2005; Foken, 2008) shown by Cabral et al.
225 (2012), who found a slope of 0.97 in the linear regression through the origin between
226 the sum of turbulent fluxes of heat and water vapor versus the available energy. Thus
227 the cospectral corrections (Moore, 1986) utilized were effective and the necessary
228 conditions for the eddy-covariance method, such as an adequate fetch (Hsieh et al.,
229 2000) of homogeneous surface, have been met (see Baldocchi, 2003). However the
230 recent results of Frank et al. (2013) showed that the use of a non-orthogonal sonic
231 anemometer (CSAT3, Campbell SI, Logan, UT, USA) can lead to 10% underestimation
232 in w ; if this is a general result it would be applicable to the sonic anemometer utilized
233 here and this assumption must be reassessed.

234 The evapotranspiration fluxes (ET) necessary to calculate the water use efficiency
235 (WUE=GEP/ET) were measured simultaneously at the site; further details are given by
236 Cabral et al. (2012).

237

238 **2.4 – Sugarcane biomass**

239

240 The aboveground biomass (stalks; green and senesced leaves) was sampled in ten
241 random plots, each 1 m along a planting line, which was equivalent to 1.4 m² of surface
242 area. Samples were taken at approximately 20-day intervals and on each harvest day,
243 i.e., 13/04/2005 (day 103); 11/05/2006 (day 131) and 20/05/2007 (day 140). Ten sub-
244 samples (10% of the fresh weight) were dried to constant weight in a forced ventilation
245 oven (60 °C) and all them were analyzed for nitrogen concentration using the Kjeldahl
246 digestion method (see Coombs et al., 1987) and for carbon by the dry combustion
247 method (see Roberts et al., 1987).

248 The mean specific leaf area was determined from the area and dry weight of leaf
249 samples collected during both cycles for green (10.2 m² kg⁻¹) and senesced (9.6 m² kg⁻¹)
250 leaves, and were used to obtain the leaf area indices of green (LAI_g) and dead (LAI_d,
251 senesced) leaves respectively from the sampled dry biomass.

252 The root-system estimate was derived from the root-to-shoot ratio (see Smith et al.,
253 1999), based on the root and aboveground dry biomass data obtained in similar
254 conditions reported by Faroni and Trivelin (2006), Otto et al. (2009) and Battie Laclau
255 and Laclau (2009), whose mean root-to-shoot ratio was 0.122±0.056 over a range from
256 0.043 to 0.220. The estimated root loss during harvest of 17% was based on the work of
257 Ball-Coelho et al. (1992). Error propagation was addressed by the accumulation
258 principle (Aurela et al., 2002) assuming the fractions to be independent (see Béziat et
259 al., 2009).

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

263

264 **3 – Results and discussion**

265

266 **3.1 – Cospectral corrections, U_* filter and the ecosystem respiration estimates**

267

268 The estimated first order sensor characteristic time (τ_s) was 0.35 s when the tubing of
269 the closed-path system was new at the beginning of the measurements and 0.65 s two
270 years later, at the end. However, applying these values of τ_s to the heat flux cospectra
271 implied CO₂ flux losses varying from 10% (daytime) to 12% (nighttime) in agreement
272 with Mammarella et al. (2009) who found a small difference in the flux correction for
273 this range of τ_s . The corrections obtained here were similar to the results previously
274 observed by Sakai et al. (2004) over grass but higher than the 4% obtained by Ibrom et
275 al. (2007) and Mammarella et al. (2009), probably a consequence of the non-turbulent
276 flow in the tubing we have utilized, as well as the greater contribution from eddies
277 associated with higher attenuated frequencies above the sugarcane.

278

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

285 U_* threshold as low as 0.1 m s^{-1} were also found in stands of crops (maize, miscanthus
286 and switchgrass) by Zeri et al. (2011), while values as low as 0.08 m s^{-1} were reported
287 by Sakai et al. (2004) over pasture and rice, and Aires et al. (2008) over a
288 Mediterranean grassland site.

289

290 In order to gapfill the nighttime fluxes we derived exponential relationships (Van 't Hoff
291 equation; see Lloyd and Taylor, 1994) between nighttime NEE data versus air
292 temperature for a range of soil water contents (Reichstein et al., 2002; Cabral et al.,
293 2011). The coefficients, given in Table 1, indicate sensitivities (Q_{10}) of the sugarcane
294 system respiration to air temperature as low, as 1.2 representing the initial crop growth
295 in the dry winter; but as high as 2.4, characteristic of the fully developed canopy in the
296 wet summer. The intermediate curves contained data covering transient conditions
297 spread over the whole period of measurement. The estimated increase in Q_{10} implicitly
298 represented the increase in biomass autotrophic respiration because the Q_{10} dependence
299 on soil moisture seasonality in fact reflected the sugarcane growth cycles and their
300 different timing within seasons due to the distinct regrowth from stubble conditions (see
301 Section 3.10.1 below). According to Mahecha et al. (2010) single-site studies of the
302 intrinsic temperature dependence of respiration can be confounded by the factors
303 seasonally co-varying with temperature.

304

305 <<Place Table 1 about here>>

306

307

308 **3.2- Gap filling and the estimated errors**

309

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
319 (Falge et al., 2001; Moffat et al., 2007). The estimated uncertainties were ± 29 and $\pm 20 \text{ g}$
320 $\text{C m}^{-2} \text{ cycle}^{-1}$, respectively and the random error (Morgenstern et al., 2004; Richardson
321 and Hollinger, 2007) produced $\pm 30 \text{ g C m}^{-2} \text{ cycle}^{-1}$. The selective systematic error
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).

326 The uncertainty of the U_* threshold detection resulted in errors of $\pm 14 \text{ g C m}^{-2}$ and ± 53
327 g C m^{-2} for each cycle, respectively. Therefore the estimated uncertainties in NEE
328 measurements and the scatter in the fitted model results for each cycle, assuming the
329 errors to be independent (Flanagan and Johnson, 2005), were ± 44 and $\pm 64 \text{ g C m}^{-2}$
330 cycle^{-1} , respectively.

331 These values are above the limits (± 20 to $\pm 40 \text{ g C m}^{-2}$) reported over different
332 agrosystems (Béziat et al., 2009; Zeri et al., 2011) and greater than the effect of gap
333 filling on the annual sums of NEE ($\pm 25 \text{ g C m}^{-2} \text{ yr}^{-1}$) given by Moffat et al. (2007),
334 although only based on forest sites.

335 We have applied the conventional steady state test which accordingly to Béziat et al.
336 (2009) can lead to data losses around zero covariance. For our 30-minute NEE data set,
337 17% were within the threshold of $\pm 1.25 \mu\text{mol m}^{-2} \text{s}^{-1}$, however as reported by Béziat et
338 al. (2009) the modified steady state test resulted in NEP differences lower than 3%.
339 The R_E error was assessed from the nighttime U_* threshold error and from the
340 associated errors of the fitted curves (SSE in Table 1) for the daylight hours utilizing the
341 accumulation principle (Aurela et al., 2002), and resulted in $\pm 14 \text{ g C m}^{-2}$ and $\pm 53 \text{ g C}$
342 m^{-2} for each cycle, respectively.

343

344 **3.3-The climate patterns**

345

346 The air temperature shown in Fig. 1a ranged from 5 to 36 °C and the vapor pressure
347 saturation deficit (VPD) from 0.1 to 5.0 kPa. The region is characterized by a relatively
348 dry winter exhibiting low rainfall and consequently lower soil water content (Fig. 1b).
349 This lack of soil water probably promoted some vegetation stress as the wilting point
350 was nearly reached in September of both years. The total rainfall recorded in each cycle
351 was 1194 and 1353 mm respectively, and although the first cycle total was lower than
352 the long term average ($1517 \pm 274 \text{ mm}$) the rainfall resulting from passing cold fronts in
353 the winter of 2005 (203 mm) was greater than the total observed in the winter of 2006
354 (34 mm). The consequences of this inter-annual variability of rainfall on the water
355 balance have already been reported by Cabral et al. (2012).

356

357 <<Place Figure 1 about here>>

358

359 The photosynthetically active radiation (PAR) and friction velocity are shown in Fig.
360 1c. The PAR fluxes show a clear seasonality whose maxima ranged from 1200 to 2300
361 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$ between winter and summer, respectively. However the intense
362 summer rainfall observed in 2007 and the associated cloudy periods reduced the level of
363 PAR fluxes by approximately 17%. U_* also exhibited a seasonal variation with a
364 maximum recorded between October and December due to the increased horizontal
365 wind speed promoted by the regional atmospheric circulation, and the nighttime values
366 observed during these months in 2006 were greater than 2005.

367

368 **3.4 – Aboveground biomass and LAI**

369

370 The measured aboveground biomass fractions and the green leaf area index are depicted
371 in Fig. 2. The observed peak in green leaf biomass was $266 \pm 22 \text{ g C m}^{-2}$ in the first cycle
372 and $209 \pm 12 \text{ g C m}^{-2}$ in the second cycle. However the peak biomass in dead leaves
373 ($619 \pm 47 \text{ g C m}^{-2}$) and stalks ($1677 \pm 113 \text{ g C m}^{-2}$) measured in the first cycle decreased
374 by approximately 43% in the second cycle and there was a delay in the canopy
375 development of approximately two months – the maximum LAI_g (5.8) in the first cycle
376 was achieved on 27/12/2005 (day 361), while in the second cycle ($\text{LAI}_g=4.6$) it was
377 recorded on 14/02/2007 (day 45).

378

379 <<Place Figure 2 about here>>

380

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

384 DAH) characterized by slower growth activity (Tejera et al., 2007). The decrease in
385 yields, commonly observed in sugarcane ratoons (see Cabral et al., 2012), is mainly a
386 consequence of the lower initial shoot population, whose unsprouted stubble promotes
387 gaps in stubble rows (Shukla et al., 2009). The gaps in ratoons arise due to mechanical
388 damage to stubble roots, from pests, diseases and nutritional limitations as well as the
389 environmental growing conditions such as the level of soil water, and the timing and
390 severity of early and mid-season water deficits (Robertson et al., 1999; Ramesh, 2000;
391 Zhao et al., 2010).

392 The dry matter partition in stalks and dead leaves during the formative phase in the first
393 cycle, were 39% and 18% respectively; while in the second cycle the same biomass
394 fractions represented 30% and 23% of the total dry matter, respectively. The reduced
395 leaf area and the senescence of older leaves are typical dehydration avoidance
396 mechanisms (Lopes et al., 2011). The early growth was dominated by the partitioning of
397 dry mass into vegetative organs (Ramesh, 2000) particularly in the second cycle;
398 however, as the crop advanced toward maturity, the same fraction of dry biomass,
399 approximately 66%, was directed to the storage organs (stalks) in both cycles.

400 Although the number of days after harvest (DAH) necessary to achieve the maximum
401 LAI_g was practically the same in both cycles: 305 and 308 DAH, respectively, the leaf
402 area duration (LAD; see Beadle, 1987; Chiariello et al., 1991) which represents the area
403 under the LAI_g curve was 1144 days in the first cycle and 717 days in the second,
404 implying in a decrease of 37% in LAD between cycles. The LAI_g integration over the
405 sugarcane main development phases (formative, grand growth and maturity) resulted in
406 14%, 46% and 40% of total LAD in the first cycle, respectively; while in the second
407 cycle the percentages were 6%, 39% and 55%, respectively. Hence in the second cycle

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

416

417 **3.5-Seasonal patterns of NEE, GEP and R_E**

418

419 The time series of daily totals of GEP and R_E , as well the cumulative NEE, are depicted
420 in Fig. 3. The GEP in the first cycle shows a steady increase from the harvest in April
421 2005 until 10/01/2006, values above $15 \text{ g C m}^{-2} \text{ day}^{-1}$ were then achieved during the
422 main growth period from December to February of 2006, after which they slowly
423 decreased – a characteristic of the maturity phase. The beginning of the second cycle
424 was marked by a slower re-growth from May to October of 2006, as the GEP increase
425 was enhanced only after the onset of the rainy season in October (Fig. 1b). The fully
426 developed canopy conditions were observed in February of 2007, when the GEP
427 reached about the same value as previously. The seasonal patterns of daily totals of
428 ecosystem respiration exhibited a dependency on GEP (see Migliavacca et al., 2011;
429 Suleau et al., 2011). Peak GEP values obtained in both cycles ($\sim 21 \text{ g C m}^{-2} \text{ day}^{-1}$) were
430 similar to the values found for maize (Béziat et al., 2009; Stella et al., 2009; Kalfas et
431 al., 2011), as was the observed range of R_E from 1 to $10 \text{ g C m}^{-2} \text{ day}^{-1}$.

432

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

444

445 <<Place Figure 3 about here>>

446

447 The monthly totals of gross ecosystem productivity (GEP), ecosystem respiration (R_E)
448 and their ratios (GEP/R_E) are presented in Fig. 4. The initial two months of the first
449 cycle exhibited R_E totals greater than GEP, but in June of 2005 the assimilation
450 exceeded the respiration; however, the GEP/R_E ratios were close to, or above, two for
451 most of the cycle. These GEP/R_E ratios around two imply that $NEP \sim R_E$ and indicate that
452 the autotrophic respiration drives the carbon fluxes, although when GEP substantially
453 exceeds R_E the system withdrawal of available nutrients is maximized (Falge et al.,
454 2002). These results confirm the importance of the biomass in the Q_{10} sensitivity as
455 discussed in Section 3.1, because in crops the large temperature sensitivities mainly
456 result from leaf assimilation and plant growth rather than being a direct R_H response to
457 temperature (Aubinet et al., 2009).

458 Contrasting with the first cycle, the sugarcane agrosystem was a source of CO₂ to the
459 atmosphere during the initial five-month period of re-growth in the second cycle
460 (GEP/R_E<1). This resulted from the sugarcane plants being smaller due to receiving less
461 rainfall (Fig. 1b) and consequently having lower rates of photosynthesis (GEP) and R_E
462 (van der Molen et al., 2011). Notice that GEP exceeded R_E only in November of 2006.
463 From the totals summarized in Table 2, the observed decreases in NEE and GEP
464 between cycles were 51% and 25%, respectively. Further, during the initial 150 days
465 (tillering phase) in the second cycle the sums of GEP and R_E were lower than in the first
466 cycle by 62% and 20%, respectively, in agreement with the results of Schwalm et al.
467 (2010), which indicated that GEP sensitivity to water stress was approximately 50%
468 greater than R_E with the agricultural areas exhibiting the highest sensitivity. The
469 respiratory costs (R_E/GEP) were considerably higher in the beginning of the re-growth
470 particularly in the second cycle, and decreased toward the final phase (see Fig. 4). The
471 total costs in each cycle were 46% and 65%, respectively, indicating that in the second
472 cycle the respiration costs were 41% greater (Table 2). The R_E/GEP observed in the first
473 sugarcane cycle was lower than the ratios reported for maize, soybean and wheat crops,
474 i.e., from 0.55–0.66 (Law et al., 2002; Suyker et al., 2005; Verma et al., 2005;
475 Moureaux et al., 2008), although the upper limit of the interval was achieved in the
476 second cycle.

477

478

479 <<Place Figure 4 about here>>

480 <<Insert Table 2 about here>>

481

482

483 **3.6 – Water use efficiency (WUE)**

484

485 Accordingly to Suyker and Verma (2010), the consequence of an extended dry period in
486 maize is reduction in GEP, as well as in the evapotranspiration (ET). The sugarcane
487 evapotranspiration reduction between cycles, reported by Cabral et al. (2012), was of
488 the same order (17%) as found here for the GEP decrease. Based on the same
489 evapotranspiration data (Cabral et al., 2012), we calculated the water use efficiency
490 (WUE) defined as the GEP/ET ratio (Suyker and Verma, 2010) over the three sugarcane
491 main phases (Table 2). The sugarcane water use efficiency was lower in the second
492 cycle during tillering (17%), grand growth (16%) and the decrease in the final phase
493 (maturation) represented the same (5%) as the overall WUE decrease between cycles
494 (totals in Table 2). However, the low WUE values obtained in the initial sugarcane
495 phase are comparable to the maximum WUE for miscanthus ($2.2 \text{ g C kg}^{-1} \text{ H}_2\text{O}$) reported
496 by VanLoocke et al. (2012) and soybean (Suyker and Verma, 2010); and the WUEs in
497 the second phase were similar to the average growing season totals for maize (3.2 g C
498 $\text{kg}^{-1} \text{ H}_2\text{O}$) given by Suyker and Verma (2010).

499

500

501 **3.7 - NPP and R_E partition**

502

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

508 249 ± 132 and 1399 ± 131 g C m⁻², respectively and in the second cycle 422 ± 112 and
509 1337 ± 99 g C m⁻², respectively. R_H increased 69% (173 g C m⁻²) in the second cycle, but
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
512 represented a loss of 105 ± 12 g C m⁻² and the likely sources were the old root system
513 turnover estimated as 45 ± 15 g C m⁻² (see below) and part of the biomass remaining on
514 the soil due to incomplete burning, an estimate of this (81 ± 73 g C m⁻²) was obtained by
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
517 labile organic carbon (van der Molen et al., 2011) resulted in 126 ± 74 g C m⁻², which did
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).

524

525 <<Place Table 3 about here>>

526

527 **3.8 – Carbon use efficiency (CUE)**

528

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

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

542

543 **3.9 – Net ecosystem carbon balance (NECB)**

544

545 In this study the net ecosystem carbon balance (NECB) is defined as the sum of NEE
546 and the carbon losses during the harvest by non-respiratory processes (Anthoni et al.,
547 2004), which in this sugarcane plantation should include the biomass burning and the
548 stalk removal (seeds and fertilizers inputs not considered here). The total dead leaf
549 biomass and a fraction of the green leaves (37%) were burnt (see Cerri et al., 2011),
550 resulting in direct carbon emissions of 630 ± 31 and 406 ± 23 g C m^{-2} in the first and
551 second cycle, respectively. Accounting for the stalk removal (1436 ± 118 and 957 ± 49 g
552 C m^{-2} , in each cycle respectively) the NECB of the sugarcane agrosystem was
553 approximately carbon neutral (102 ± 130 g C m^{-2}) in the first cycle due to the large
554 uncertainty, and a carbon source (403 ± 83 g C m^{-2}) in the second cycle, as a
555 consequence of the management practices as well the water shortage observed at the
556 beginning of the second cycle.

557

558 The NECB represents the changes in non-harvestable carbon pools as the soil organic
559 carbon, root biomass and aboveground residue (Anthoni et al., 2004; Verma et al.,
560 2005; Zeri et al., 2011). The amount of sugarcane residue remaining aboveground after
561 the first cycle harvest, due to the incomplete burning was 159 ± 73 g C m⁻² and the
562 estimated root biomass turnover was 45 ± 15 g C m⁻², therefore the sum of these
563 heterotrophic respiration sources represented 204 ± 74 g C m⁻², which subtracted from
564 NECB resulted in 199 ± 112 g C m⁻² originating from the soil pool. This estimate
565 assumes that 100% of the aboveground residues had decomposed at the end of the
566 second cycle, which is of course an overestimation (see Robertson and Thorburn, 2007).
567 There are also great uncertainties associated with the estimated root system. The results
568 of root biomass reported here were based on samples taken to a maximum depth of 1.0
569 m. Battie Laclau and Laclau (2009) indicated that 50% of the root intersects (see
570 Chopart et al., 2008) were below 1 m depth (see Cabral et al., 2012); therefore the
571 average ratio of 0.122 is probably an underestimate of the real root-to-shoot ratio as
572 well as the root turnover figure (17%) given by Ball-Coelho et al. (1992). Denmead et
573 al. (2010b), working in Australia, estimated that the sugarcane root biomass represented
574 32% of the harvested biomass, if we suppose this figure represents the sugarcane in
575 Brazil as well, and applying it to the first cycle aboveground net primary productivity
576 (ANPP in Table 3) the root biomass and turnover would decrease the loss of carbon
577 originating from the soil by approximately 50%. The integrated soil organic carbon
578 obtained after the harvest in 2007 was 2880 ± 340 g C m⁻² in the first 0.3 m of soil and
579 6350 ± 500 g C m⁻² down to 1 m depth. Therefore the estimated emissions of 200 g C m⁻²
580 would represent 7% to 3% of the soil carbon stock depending on the soil layer
581 thickness. Temporal changes in soil carbon are small and difficult to detect by soil
582 sampling in the short term (Verma et al., 2005) as they exhibit large coefficients of

583 variation. Changes in soil carbon of approximately $100 \text{ g C m}^{-2} \text{ yr}^{-1}$ 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 $138 \pm 239 \text{ g C m}^{-2} \text{ yr}^{-1}$ (Robertson et al, 2000; Ceschia et al.,
591 2010; Ciais et al., 2010). However other crop studies report carbon losses of $95 \pm 87 \text{ g C}$
592 $\text{m}^{-2} \text{ yr}^{-1}$, 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 ($100 \text{ g C m}^{-2} \text{ yr}^{-1}$) 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 ($96 \text{ g C m}^{-2} \text{ yr}^{-1}$), 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 $200 \text{ g C m}^{-2} \text{ yr}^{-1}$. 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.

602

603 **3.10 - Environmental controls**

604

605 **3.10.1 - GEP and R_E dependency on LAI_g**

606

607 The scatter plot between daily means of half-hour GEP averaged when PAR was above
608 $1500 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$ versus LAI_g (Fig. 5a) indicates that LAI_g accounted for more
609 than 90% of the GEP variability at light saturation in each cycle, in agreement with
610 other crops and grassland studies where LAI_g usually explains above than 80% of the
611 GEP variance (Flanagan et al., 2002; Xu et al., 2004; Aires et al., 2008; Suyker and
612 Verma, 2010; Polley et al., 2011). The average GEP increases were 9.9 and 11.7 μmol
613 $\text{CO}_2 \text{ m}^{-2} \text{ of leaf s}^{-1}$ in each cycle, respectively per unit of LAI_g increase, therefore the
614 slope of the GEP and LAI_g relationship was 18% greater in the second cycle, despite the
615 lower yield (see Table 3). Roberts et al. (1990) have already noticed this effect in
616 sugarcane as the compensatory growth after re-watering, because photosynthesis is less
617 sensitive to soil water limitations than the crop growth (Polley et al., 2011). Although
618 the recovery is partial (Ghannoum, 2009), it may contribute to the attenuation of the
619 initial water stress effects on the final yield depending on the climate conditions towards
620 the end of the cycle and if it is extended, with the application of ripeners and harvest
621 postponed, as for example observed by Cabral et al. (2003) who reported similar sugar
622 cane yields (107 t ha^{-1}) in a more productive area, at the end of the fourth and sixth
623 ratoons, mainly because the latter cycle was 40 days longer.

624

625 <<Place Figure 5 about here>>

626

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

632 summer temperatures mediated by the soil moisture content (Davidson et al., 2006)
633 whose lower values observed during the initial stages of re-growth reduced the total
634 respiration from biomass and soil. Notice that R_E was on average approximately 3.5 g C
635 $m^{-2} day^{-1}$ greater in the second cycle for the same LAI_g (Fig. 5b), therefore precluding
636 the estimation of R_E based in LAI_g intervals as reported for other agrosystems (Suyker
637 et al, 2004).

638

639

640 **3.10.2 - GEP versus PAR and VPD**

641

642 The relationships between half-hour ensembles of GEP and PAR, calculated over LAI_g
643 intervals are depicted in Fig. 6 for both cycles, as well as the fitted rectangular
644 hyperbolas ($GEP = \alpha GEP_{max} PAR / (GEP_{max} + \alpha PAR)$). The curves were similar for
645 $1 < LAI_g < 2$ (Fig. 6a) and probably exhibit the effect of incomplete absorption of the light
646 by the plants as the canopy was not fully-closed (Valentini et al., 1995). However
647 contrary to expectation, the initial slope of the light curve which represents the apparent
648 photon yield (α) and the asymptotic value (GEP_{max}) in the $2 < LAI_g < 3$ and $3 < LAI_g < 4$
649 intervals (Fig. 6b) were greater in the second cycle, although when nearly complete
650 canopy cover was achieved (see Shuttleworth and Gurney, 1990). For the second cycle,
651 in the $3 < LAI_g < 4$ the light curve (Fig. 6c) exhibited afternoon depression (Hirasawa and
652 Hsiao, 1999; Xu et al., 2009; Lasslop et al., 2010), whilst no signs of saturation were
653 observed in the first cycle.

654 Notwithstanding, the α range found, i.e., from 0.03 to 0.07 mol CO_2 mol $^{-1}$ quanta, was
655 similar to the values obtained for maize (Suyker et al., 2004 and 2005; Béziat et al.,
656 2009; Arkebauer et al., 2009); as well as the GEP_{max} (from 8 to 53 $\mu mol CO_2 m^{-2} s^{-1}$) for

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

660

661 <<Place Figure 6 about here>>

662

663 The growth cycles occurred at different times of the years (see Fig. 2) as noticed from
664 the higher PAR (Fig. 6a) over the interval $1 < LAI_g < 2$ in the second cycle because it
665 covered the period October–November of 2006 (days 291-328) while in the first cycle it
666 was observed in July–August of 2005 (days 186-233), hence for the other LAI_g intervals
667 depicted in Fig. 6 (b-c) the first cycle also occurred earlier in the year. The GEP and
668 PAR relationships therefore probably include the effects of other driving variables. We
669 calculated the half-hourly averages of GEP normalized by PAR bins (see Stella et al.,
670 2009); these were then plotted versus saturation deficits (VPD), covering the same
671 intervals of LAI_g already used. These results are depicted in Fig. 7.

672

673 <<Place Figure 7 about here>>

674

675 The sugarcane experienced higher humidity deficits in the first cycle for $LAI_g > 2$ (Fig.
676 7b), somewhat explaining the observed patterns in the light curves for $LAI_g < 2$ (Fig. 6
677 b,c). However the GPP/PAR ratios were relatively less sensitive to VPD in the first
678 cycle as the ratios in the second cycle exhibited a sharp decrease with VPD, despite the
679 smaller range. Therefore, the GEP decreases observed in the sugarcane mainly resulted
680 from the high atmospheric demand for water exceeding soil-plant hydraulic system (see
681 Lizaso et al., 2005) and the consequent stomatal limitation as found by Cabral et al.

682 (2012). These authors have previously reported an exponential decrease in stomatal
683 conductance with VPD for this sugarcane plantation. According to Fletcher et al. (2007)
684 decreases in the radiation use efficiency with increasing VPD found in corn and
685 sorghum were consistent with the afternoon depression of photosynthesis. Notice that in
686 the first cycle $LAI_g=4$ was achieved at 211 days after harvest (9/09/2005, day 313)
687 against 275 days in the second cycle (10/02/2007; day 41), during the maturation phase
688 characterized by lower physiological rates (Ramesh, 2000; Tejera et al., 2007; van
689 Heerden et al, 2010). The extrapolation of the fitted curves (Fig. 7b, c) representing the
690 second sugarcane cycle (gray lines) towards the deficits experienced in the first cycle
691 would produce average decreases in the GEP/PAR ratios of approximately 50%.
692 Thus physiological effects on transpiration, photosynthesis and respiration (Zhao et al.,
693 2010) are likely to become more common as sugarcane is increasingly grown on sandy
694 soils, where yields tend to be more vulnerable to environmental stresses such as nutrient
695 and water deficits (Ezenwa et al., 2005; Silva et al., 2007).

696

697 **3.10.3 - GEP and SLN**

698

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

705

706 <<Place Figure 8 about here>>

707

708 The overall range of SLN, from 0.7 ± 0.03 to 1.7 ± 0.13 g N m⁻² of leaf is within the
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
714 and Lemaire, 2002; McCormick et al., 2008). The values around 15 μmol CO₂ m⁻² of
715 leaf s⁻¹, which represent the canopy integrated GEP, are comparable to the whole plant
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

732 survival of the stool and growth of the following ratoon crop (Smith et al., 2005).
733 Results from sugarcane model simulations on adaptation to drought (see Inman Bamber
734 et al., 2012) indicate that increased rooting depth results in an up to 21% increase in
735 mean dry biomass yield depending on the climate and soils, but the spraying of
736 chemical ripeners (Inman-Bamber et al., 2008; Lopes et al., 2011), which is a common
737 pre-harvest practice in sugarcane, can also adversely impact the ratooning, as the early
738 induced maturation reduces the level of photo-assimilates partitioned into the root
739 system (Viator et al., 2010).

740

741

742 **4-Conclusions**

743

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

752 Our results also show that the NECB measured above the sugarcane plantation was
753 approximately carbon neutral ($102 \pm 130 \text{ g C m}^{-2} \text{ yr}^{-1}$) in a normal cycle (second ratoon),
754 whose yield attained the expected regional average. However, it was a carbon source
755 ($403 \pm 83 \text{ g C m}^{-2} \text{ yr}^{-1}$) in the following cycle (third ratoon) when the yield was 19%
756 lower than the regional average and 26% lower than the previous ratoon. The carbon

757 losses resulted mainly from harvest but also the reduced re-growth resulting from the
758 lower rainfall during the initial 150 days of the second cycle, when the ecosystem
759 respiration (mainly heterotrophic) was greater than the assimilation due to the prolonged
760 period with partially covered soil. Although the plantation re-established the growth
761 after the onset of the summer rainfall, the assimilated CO₂ did not offset the net carbon
762 emissions associated with the stubble burning and stalk removal.

763 For the conditions observed here, our results indicate that the avoidance of the carbon
764 released during burning would lead to a negative (sink) net ecosystem carbon balance in
765 the first cycle and a neutral balance in the second cycle. In approximately 40% of the
766 total area of sugarcane in Brazil, pre-harvest burning is not used and the trash remains
767 on the soil surface, although most of the trash decomposes during the cycle it is
768 generally assumed that a change in farming practice from burning to trash conservation
769 will have benefits for cane productivity and increase soil carbon stocks. Our results
770 stress the importance of implementing a biomass burning ban, which must be pursued
771 by the Brazilian government and industry.

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784

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1349 **Figures captions**

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1352 Figure 1- Time series of 30-minute averages/totals of (a) air temperature (black lines)
1353 and water vapor saturation deficit (VPD, gray dashed lines); (b) rainfall (black lines)
1354 and soil water content in the first meter of soil (gray line); (c) photosynthetically active
1355 radiation (PAR, black area) and friction velocity (U_{*} , gray area).

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1357 Figure 2- Aboveground biomass: green leaves (circles); dead leaves (squares) and stalks
1358 (triangles). The green leaf area index (LAI_g) is represented by the gray line. The error
1359 bars represent \pm standard deviation.

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1361 Figure 3 – Daily totals ($g\ C\ m^{-2}\ day^{-1}$) of gross ecosystem productivity (GEP, thick
1362 black line), ecosystem respiration (R_E , gray line) and the cumulative net ecosystem
1363 exchange (NEE, thin black line) above the sugarcane plantation. The horizontal gray
1364 arrows indicate the main phases duration of the cycles (see text) and the black bar the
1365 time of the first cycle harvest (11/05/2006, day 131).

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1367 Figure 4 - Monthly totals of gross ecosystem productivity (GEP, gray bars), ecosystem
1368 respiration (R_E , black bars) and their ratios (GEP/R_E , transparent bars) observed during
1369 the two cycles of sugarcane.

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1371 Figure 5 – Scatter plots of (a) daily mean gross ecosystem productivity (GEP, $\mu mol\ CO_2$
1372 $m^{-2}\ s^{-1}$) averaged when $PAR > 1500\ \mu mol\ quanta\ m^{-2}\ s^{-1}$ and (b) daily totals of ecosystem
1373 respiration (R_E , $g\ C\ m^{-2}\ day^{-1}$) versus green leaf area index (LAI_g , $m^2\ m^{-2}$) during the
1374 first (black circles) and second (gray squares) sugarcane cycles.

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1376 Figure 6 – Scatter plots of half-hourly ensembles of gross ecosystem productivity (GEP,
1377 $\mu mol\ CO_2\ m^{-2}\ s^{-1}$) versus photosynthetic active radiation (PAR, $\mu mol\ quanta\ m^{-2}\ s^{-1}$)
1378 calculated over green leaf area index intervals: (a) $1 < LAI_g < 2$; (b) $2 < LAI_g < 3$; (c)
1379 $3 < LAI_g < 4$. The lines represent the fitted hyperbolas in the first (black circles and lines)
1380 and second (gray squares and lines) sugar cane cycles. The error bars represent the
1381 \pm standard deviations.

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1383 Figure 7 – Scatter plots of gross ecosystem productivity (GEP) normalized by the
1384 photosynthetic active radiation (PAR) bins versus water vapor saturation deficits (VPD,
1385 kPa) calculated over green leaf area index intervals: (a) $1 < LAI_g < 2$; (b) $2 < LAI_g < 3$; (c)
1386 $3 < LAI_g < 4$, during the first (black circles) and second (gray squares) sugarcane cycles.
1387 The bars represent +standard deviation due to the ordinate log scale.

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1389 Figure 8 –Time series of (a) green leaves specific leaf nitrogen (SLN) and (b) mean
1390 GEP/LAI_g ratios calculated for $PAR > 1000\ \mu mol\ quanta\ m^{-2}\ s^{-1}$ during the first (black
1391 circles) and second (gray squares) sugarcane cycles plotted as a function of the days
1392 after harvests (day 103/2005 and 132/2006, respectively). The error bars represent the \pm
1393 standard deviations.

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1399 Table 1 – Exponential relationships between night NEE (=R_E) and T_{air} (R_E = a exp^{bT_{air}})
 1400 for different intervals of soil water content (SWC, mm) in the 0-0.9 m layer. The
 1401 coefficients are followed by the standard errors (± SE). The standard error of the
 1402 estimate is represented by ± SEE and the number of degrees of freedom by DF. The
 1403 significance is indicated by: ^cP(>|t|) < 0.001 and ^dP(>|t|) < 0.05. Q₁₀ (=exp^{10b}) is the
 1404 factor by which the R_E is multiplied when T_{air} increases by 10°C.
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SWC intervals (mm)	a	b	± SSE (μmol CO ₂ m ⁻² s ⁻¹)	R ²	Q ₁₀	DF
< 100	1.0097±0.082 ^d	0.0196±0.003 ^c	1.054	0.66	1.22	91
100 -120	1.0532±0.048 ^d	0.0434±0.002 ^c	1.058	0.88	1.54	157
120-140	1.0368±0.033	0.0636±0.002 ^c	1.058	0.91	1.89	256
140-160	1.0593±0.054	0.0725±0.003 ^c	1.062	0.87	2.06	249
>160	1.0390±0.049	0.0882±0.002 ^c	1.057	0.90	2.42	86

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1440 Table 2 - Cumulative fluxes and associated uncertainties (g C m^{-2}) observed during the
 1441 two cycles of sugarcane plantation calculated over the three main phases as indicated by
 1442 the number of days after harvest (DAH) intervals. The water use efficiency
 1443 ($\text{WUE}=\text{GEP}/\text{ET}$) was calculated based on the evapotranspiration data (mm day^{-1}) from
 1444 Cabral et al. (2012).

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Cycles (DAH)	NEE	GEP	R_E	WUE
	g C m^{-2}			$\text{g C kg}^{-1} \text{H}_2\text{O}$
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

1447 Sugarcane phases: ¹Tillering, ²Grand Growth and ³Maturation.

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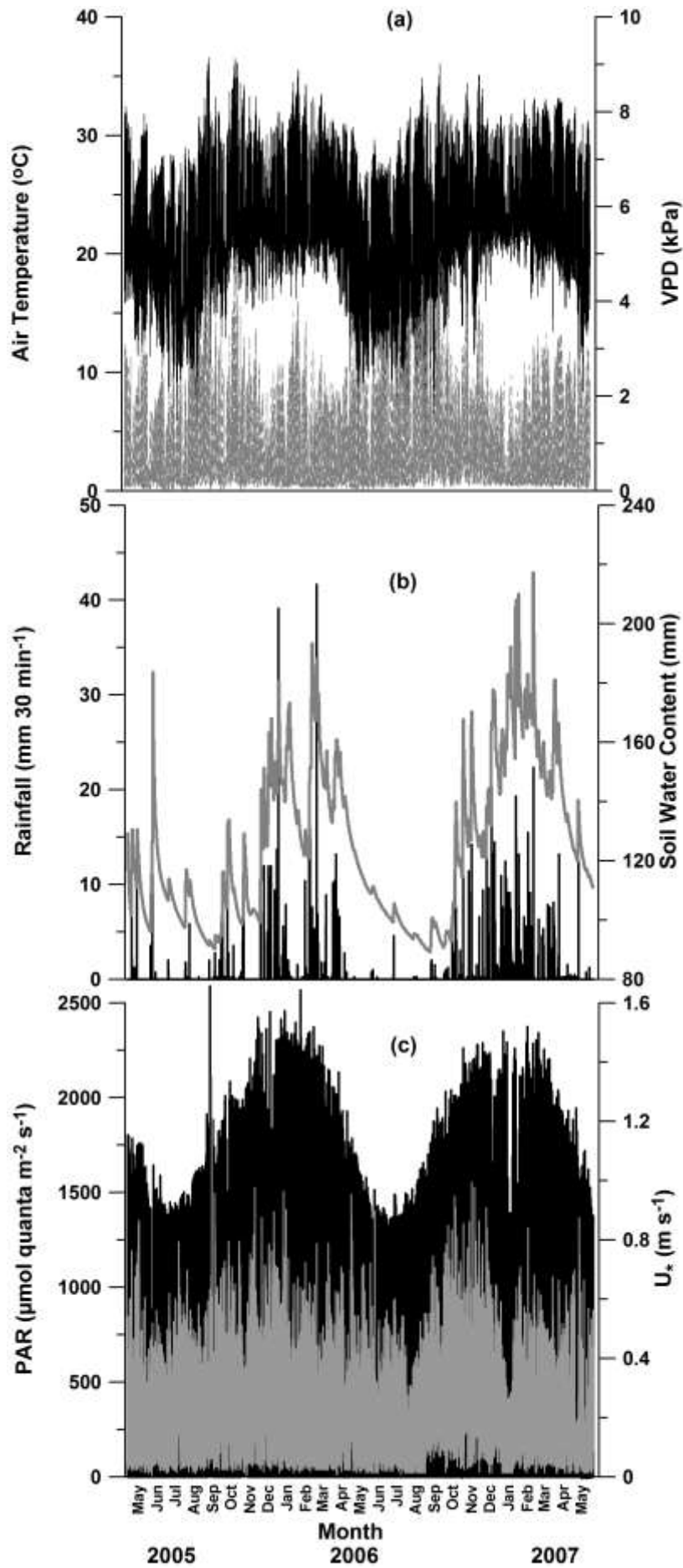
Table 3 – Components of sugarcane dry biomass (g C m⁻²) measured and estimated at the harvests. The ± represents the standard error of the mean (10 samples).

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
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

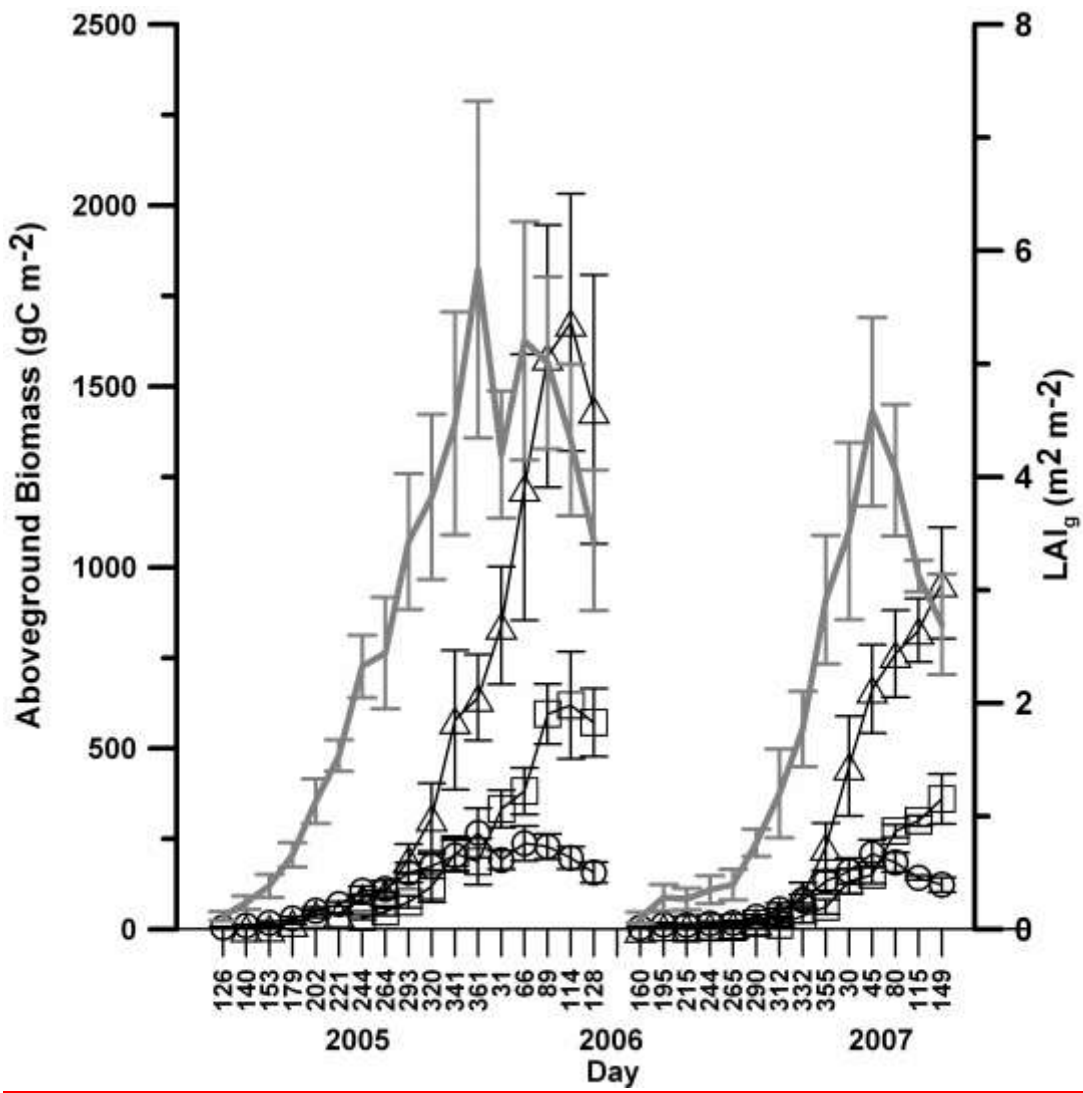
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¹ We suppose the decay in the dead leaves was negligible.

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%).

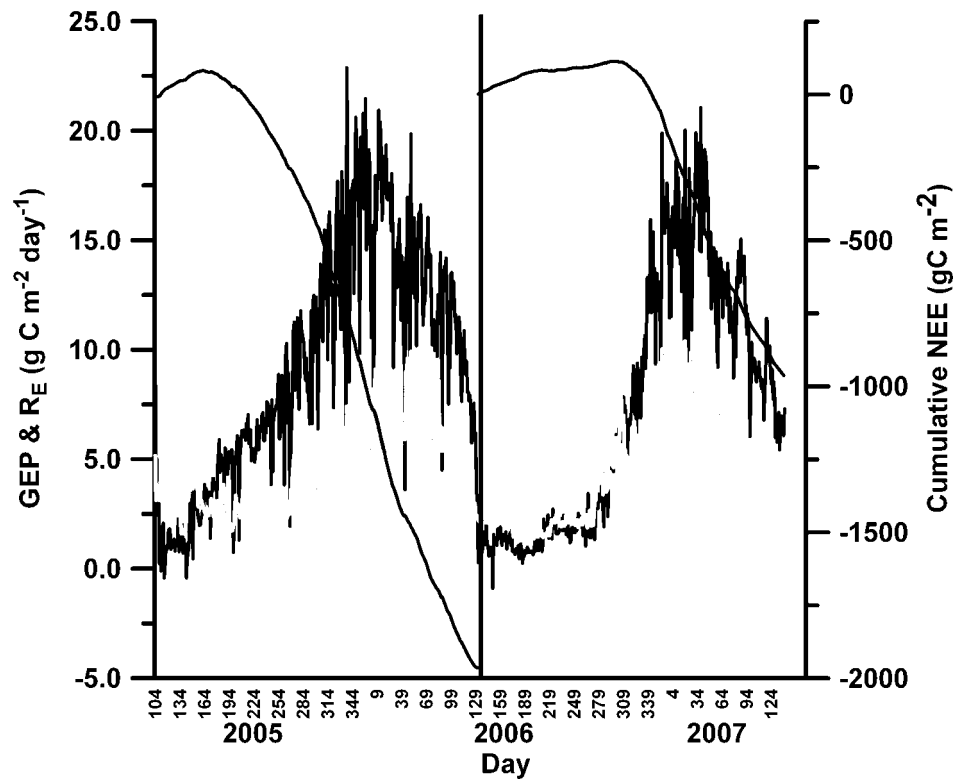


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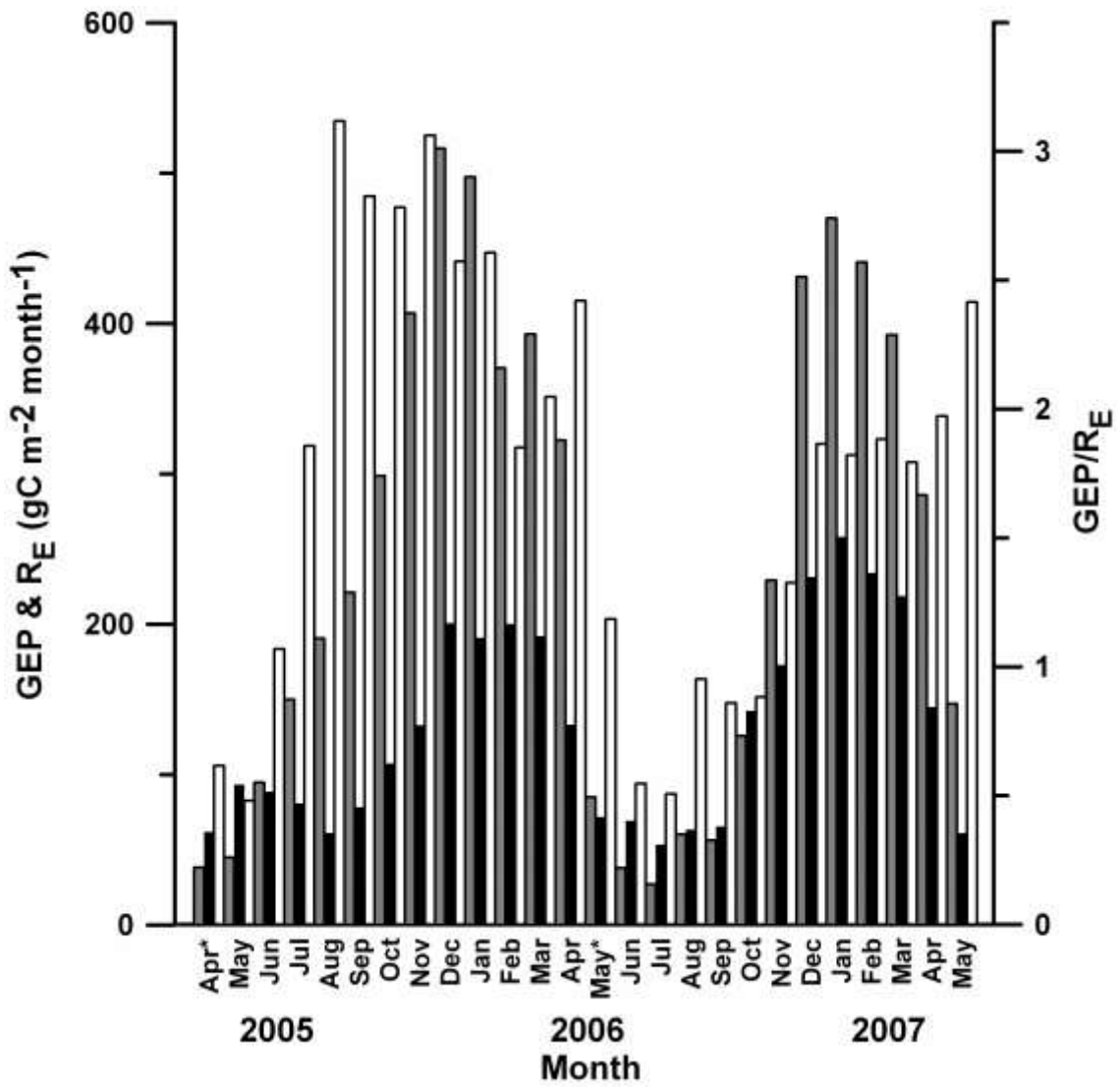
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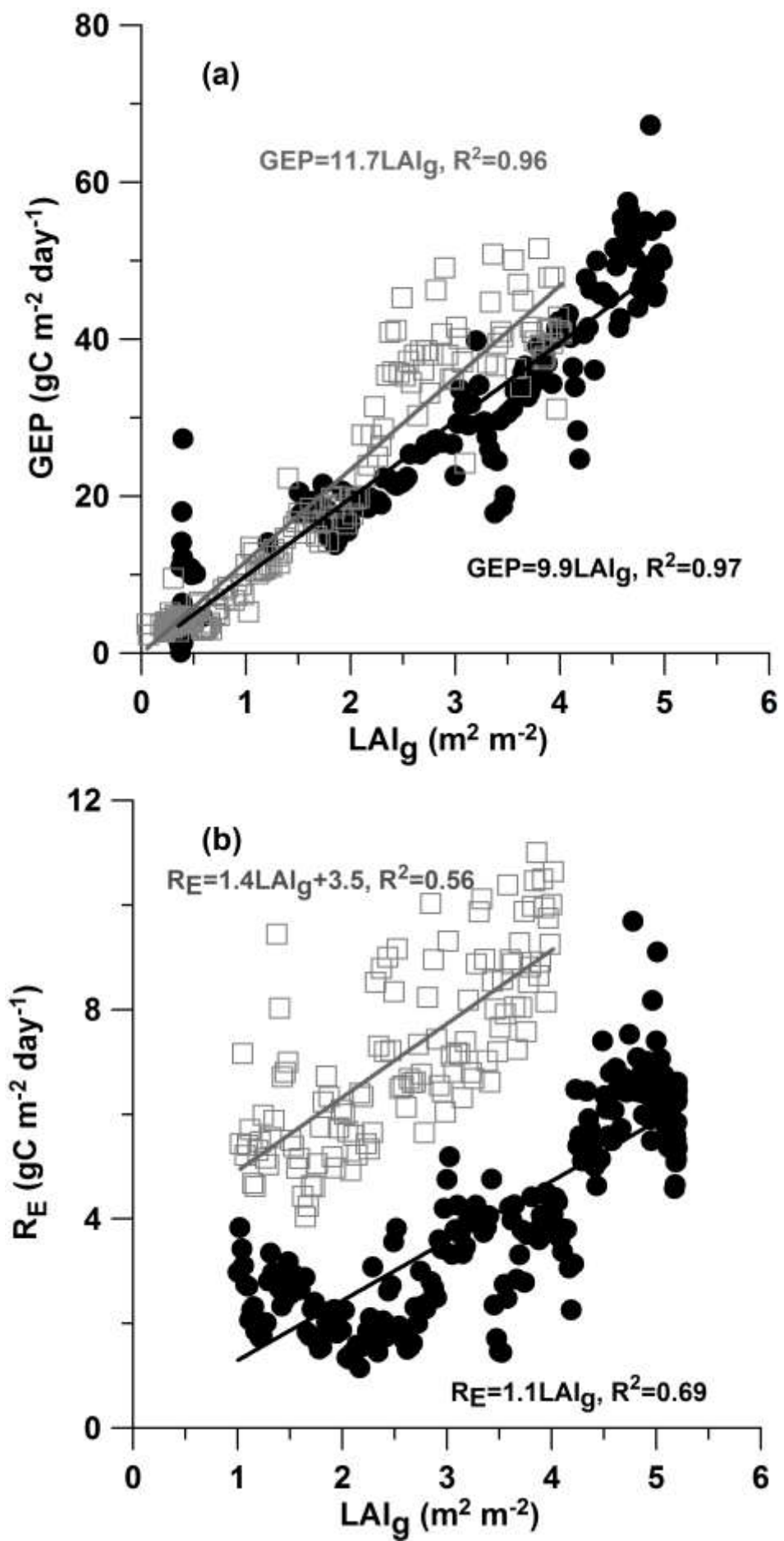
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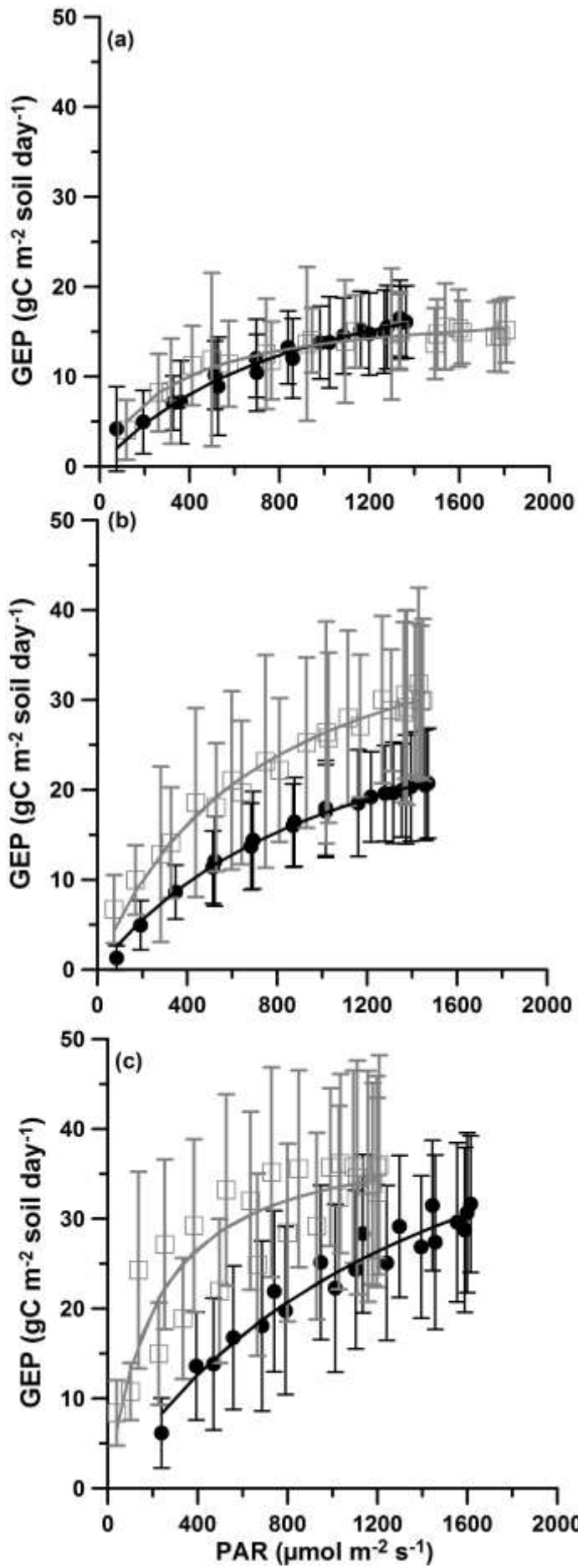
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