1 Pyrogenic carbon from tropical savanna burning: production and stable isotope

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19 Widespread burning of mixed tree-grass ecosystems represents the major natural 20 locus of pyrogenic carbon (PyC) production. PyC is a significant, pervasive, and yet 21 poorly understood 'slow-cycling' form of carbon present in the atmosphere, 22 hydrosphere, soils and sediments. We conducted sixteen experimental burns on a 23 rainfall transect through northern Australian savannas with C₄ grasses ranging from 24 35 to 99% of total biomass. Residues from each fire were partitioned into PyC and 25 further into recalcitrant (HyPyC) components, with each of these fluxes also 26 partitioned into proximal (>125µm) likely to remain close to the site of burning, and 27 distal (<125µm) likely to be transported from the site of burning. The median [range] 28 PyC production across all burns was 16.0 [11.5]% of total carbon exposed (TCE), 29 with HyPyC accounting for 2.5 [4.9]% of TCE. Both PyC and HyPyC were 30 dominantly partitioned into the proximal flux. Production of HyPyC was strongly 31 related to fire residence time, with shorter duration fires resulting in higher HyPyC vields. The carbon isotope (δ^{13} C) compositions of PyC and HyPyC were generally 32 33 lower by 1-3‰ relative to the original biomass, with marked depletion up to 7‰ for grasslands dominated by C₄ biomass. δ^{13} C values of CO₂ produced by combustion 34 was computed by mass balance and ranged from ~0.4 to 1.3‰. The depletion of ^{13}C 35 36 in PyC and HyPyC relative to the original biomass has significant implications for the interpretation of δ^{13} C values of savanna soil organic carbon and of ancient PyC 37 preserved in the geologic record, as well as for global ¹³C isotopic disequilibria 38 39 calculations.

40

41 **1 Introduction**

42 Pyrogenic carbon (PyC) describes carbon (C) in a continuum of thermally altered 43 materials produced by incomplete biomass combustion and ranging from partly-44 charred organic matter to condensed polyaromatic compounds (Hammes et al., 2007; 45 Masiello, 2004). Components of the PyC continuum have been referred to by a 46 variety of other terms, including soot, char and black carbon (Bird and Gröcke, 1997; 47 Seiler and Crutzen, 1980). This range of names reflects the compositional complexity 48 of PyC and the wide array of analytical methods employed for its quantification 49 (Hammes et al., 2007; Ascough et al., 2009). While there is wide recognition of the 50 large relevance of PyC affecting environmental processes (Bird et al., 1999; Cope and 51 Chaloner, 1980; Lehmann et al., 2008; Zimmermann et al., 2012), it remains a poorly 52 understood component of the global carbon cycle (6). Estimates of global PyC 53 production rates have varied considerably since first reported (Seiler and Crutzen, 54 1980). The difficulty in establishing a reliable estimate of PyC production rates arises 55 from the range of definitions used for PyC, usually operationally defined by the 56 analytical technique used, and also by widely divergent experimentally-derived 57 estimates of PyC production (Forbes et al., 2006). Calculations of the size of a global 58 atmospheric C sink to PyC are also complicated because some components of PyC 59 appear to be susceptible to degradation on comparatively short timescales (Bird et al., 60 1999; Zimmermann et al., 2012) while some are resistant to degradation, remaining in 61 soils and sediments for thousands to millions of years (Cope and Chaloner, 1980; 62 Lehmann et al., 2008; Masiello and Druffel, 1998). While the use of different 63 techniques does indeed lead to different results, it has become quite apparent over the 64 years that the PyC continuum is also associated to a PyC degradation continuum (Bird 65 et al., 2015).

66 Of the total PyC produced by biomass burning, it has been estimated that >90%remains (initially) close to the site of production and <10% is emitted to the 67 68 atmosphere as aerosols (Kuhlbusch et al., 1996), with much of the PyC emitted to the 69 atmosphere being ultimately deposited in the oceans (Bird et al., 2015). PvC that 70 remains on the ground will potentially (i) be re-combusted in subsequent fire events 71 (Saiz et al., 2014; Santín et al., 2013) (ii) be re-mineralized by biotic/abiotic processes 72 (Saiz et al., 2014; Santín et al., 2013; Zimmerman, 2010; Zimmermann et al., 2012), (iii) be remobilized by bioturbation, wind or water in either particulate (Major et al., 73 74 2010; Rumpel et al., 2006) or dissolved form (Dittmar, 2008; Dittmar et al., 2012) 75 and/or (iv) accumulate in the soil organic carbon (SOC) pool (Lehmann et al., 2008).

76 With fire return intervals of 5 years or less (Furley et al., 2008), tropical savannas 77 are major loci of global annual PyC production. Forbes et al. (2006) estimated the annual PyC production rate from savanna fires to be 4-40 Tg yr⁻¹ (of a global total of 78 50-270 Tg yr⁻¹), assuming 1-2% of carbon exposed to fires was converted to PyC. 79 Van der Werf et al. (2010) estimated that 39% of a total of 2.1 Tg yr⁻¹ PyC emissions 80 81 to the atmosphere derived from the burning of tropical savannas and grasslands 82 (including extra-tropical grasslands; http://www.falw.vu/~gwerf/GFED/GFED3/tables/emis BC absolute.txt), 83 whereas, 84 Bond et al. (2004) estimated savanna burning at 51% of total 'open-burning' emissions. 85

In tropical savannas, woody vegetation uses the C₃ photosynthetic pathway (δ^{13} C < -24%), whereas the grasses primarily use the C₄ photosynthetic pathway (δ^{13} C values > -15‰) (O'Leary, 1988). Randerson et al. (2005) estimated that 31% of global fire emissions have a C₄ origin and that 20% of total C₄ biomass returns to the 90 atmosphere each year as a result of fire. The production of PyC from C₃ biomass generally has little impact on the δ^{13} C of the PyC relative to the precursor biomass (< 91 92 ~1‰; Krull et al., 2003; Das et al., 2010). However, Krull et al. (2003) found that δ^{13} C values of PyC from C₄ grasses were 1.5-5‰ lower than precursor biomass. Das 93 et al. (2010) also reported PyC products with δ^{13} C values lower than original biomass, 94 but noted a difference between 'smoke' (δ^{13} C value lower by 0.5-7.2‰, species 95 dependent), and 'ash' (generally lower by up to 3.5%). Therefore, while δ^{13} C values 96 97 provide a useful tracer of the fate of tree and grass-derived PyC both during and after formation, the robust interpretation of $\delta^{13}C$ values requires a more nuanced 98 understanding of the processes controlling fractionation effects. 99

At the global scale, models of terrestrial ¹³C discrimination suggest about one 100 101 quarter of the gross primary productivity (GPP) of the terrestrial biosphere is attributable to the C₄ photosynthetic pathway (Lloyd and Farquhar, 1994; Still et al., 102 103 2003). In contrast, recent work has suggested the fraction of C₄-derived biomass in 104 SOC in savanna systems is much lower than GPP estimates would imply, partly due 105 to C₄-derived C decomposing faster than C₃-derived C in the soil (Wynn and Bird, 106 2007; Wynn et al., 2006). However, it is also plausible that during savanna burning (i) 107 C_4 carbon is preferentially combusted relative to C_3 woody biomass and/or (ii) C_4 108 biomass produces finer PyC particles than C₃ woody biomass, and thus C₄-derived 109 PyC is more likely to be exported by wind, leading to a relative accumulation of C₃-110 derived PyC at the site of burning (and ultimately in the soil), with C₄-derived PyC 111 preferentially accumulating in sites remote to burning and particularly in lacustrine sediments and the ocean (Bird and Cali, 1998). Differences between the δ^{13} C values 112 of C released to the atmosphere vs. δ^{13} C values of the C as it was fixed, or vs. the 113

114 δ^{13} C value of the SOC pool, are referred to as 'isotopic disequilibria' in mass balance 115 models of atmospheric CO₂ (Alden et al., 2010; Enting et al., 1995). In savannas, 116 large disequilibria are largely due to differences in the residence time of C₃ and C₄ 117 biomass (Randerson et al., 2005; Ciais et al., 1999; Ciais et al., 2005; Buchmann and 118 Ehleringer, 1998).

119 Quantification of the contribution of savanna fires to terrestrial isotopic disequilibrium due to potential differences in the residence times of C₃- and C₄-120 121 derived C in vegetation and soil, is important for (i) correctly interpreting soil and palaeosol carbon isotope data (Cerling et al., 2011), (ii) informing modelling studies 122 123 that use variations in the CO₂ δ^{13} C record to apportion sources and sinks of CO₂ 124 (Randerson et al., 2005; Ciais et al., 1999; Ciais et al., 2005), and (iii) enabling an 125 assessment of physical redistribution biases that might complicate the interpretation of the δ^{13} C record of PyC in terrestrial, lacustrine and marine sedimentary records (Bird 126 127 and Ascough, 2012).

128 In this paper we use a series of controlled field burning experiments in four 129 savanna environments of northeastern Australia, where the strong climatic gradient 130 exerts a major influence on vegetation structure existing across this large region 131 (Table 1, Figs. 1-2). The experiments were designed to quantify the production and 132 fate of PyC during savanna burning. Specifically, we seek to determine (i) the 133 proportion of savanna biomass that is converted to PyC during burning, (ii) the 134 distribution of this PyC between different size classes and (iii) the proportion of PyC 135 between that fraction remaining on the ground and that exported as fine particulates 136 during burning.

137 Our study takes advantage of controlled conditions, and a purpose-built field 138 apparatus designed to determine the stocks and fluxes of PyC from fires occurring in a 139 variety of savanna types (Fig. 3, see section 2.2). In addition, we used mass and 140 carbon isotope balances to test the hypothesis that there is a 'savanna isotope 141 disequilibrium effect' (SIDE) during burning, which leads to (i) an overall more negative δ^{13} C values for PyC produced in savanna fires due to the more complete 142 combustion of C₄ biomass (and hence more positive δ^{13} C values for CO₂ of 143 combustion produced in savanna fires), and (ii) an additional decrease in the $\delta^{13}C$ 144 145 value of PvC remaining at the site of production, due to the preferential export of fine C₄-derived PyC. 146

147 Because PyC represents a continuum of materials of various degradabilities, we 148 focus on the quantification of the refractory PyC component (HyPyC, polyaromatic 149 ring number >7; see methods for full definition), likely to have at least a centennial 150 residence time in the environment. Indeed, an accurate determination of the 151 abundance and stable isotope composition of the HyPyC component formed in newly 152 produced PyC is critical both for achieving an improved understanding of PyC 153 dynamics, and for better interpreting records of biomass burning, climate and 154 vegetation change in the past (Bird et al., 2015; Wurster et al., 2012). Here, we use the 155 term PyC to describe all post-fire carbon, which in our experiments might also include non-thermally altered material. 156

157 2 Materials and Methods

158 2.1 Study sites

159 The study sites were located across four contrasting savanna-grassland 160 ecosystems in Queensland (Australia). The sites extended from the drier inner region 161 to the more humid environments occurring near the northeast coast (Fig 1). The 162 different climatic conditions prevailing at the various locations strongly influenced 163 both the species and structural composition of these ecosystems. While there are 164 many definitions of 'savanna' (Torello-Raventos et al., 2013; Domingues et al., 2010) 165 the functional definition used for the purpose of this study is that of a biome 166 consisting of a continuous graminoid component coexisting with woody plants at 167 varying densities. The regional classification of vegetation was done according to 168 Torello-Raventos et al. (2013). We chose a wide climatic range in order to provide the 169 experiment with a broad spectrum of woody vs. grass biomass proportions (woody is equivalent to C_3 and grass to C_4). Indeed, there was a noticeable trend both in total 170 171 biomass and in the relative contribution of woody (C_3) vegetation to the total biomass 172 across the climatic gradient (Tables 1-2). The transect spanned dry Mitchell grasslands (site = MIT; MAP = 435mm) characterised by >97% C₄ grasses, to more 173 174 humid tall savanna woodlands (site = DCR; MAP = 2050mm). The tall savanna 175 woodlands exhibit a larger net contribution of 50-60% C₃ vegetation to the total biomass. While C₄ biomass was relatively constant across all sites, woody biomass 176 177 increased with MAP (Fig. 2, Tables 1-2). Tree canopy cover was determined by 178 means of site-specific allometric equations and visual estimates (Torello-Raventos et 179 al., 2013; Domingues et al., 2010), and ranged from <5% for a site established in a 180 heavily dominated grassland ecosystem (Mitchell grassland-MIT) to 55% for a tall 181 savanna woodland occurring at about 20 km from the coast (Davies Creek National 182 Park-DCR) (Fig 1).

The biomass present at all the fire experiment areas was mainly composed of a
grass layer dominating with sparse *Eucalyptus* and *Acacia* tree seedlings with variable

amounts of coarse woody debris and leaf litter. The most abundant grass species were

186 Themeda australis, Imperata cylindrica and Heteropogon contortus.

187 **2.2 Field methods**

188 2

2.2.1 Plot setup and pre-burn sampling

189 The experimental setup was designed to provide an estimate of initial total organic C, PyC abundance and δ^{13} C values, serving thus as reference to compare 190 191 against post-burn samples. Burning experiments were carried out on a small scale $[m^2]$, and were conceived to ensure the capture of all particulates to enable a full 192 193 isotope and mass balance. As such, the experiments were not explicitly designed to 194 simulate all characteristics of a 'natural' burn, but given the methodological issues surrounding all such techniques, the estimates of PyC production derived from this 195 196 work are unlikely to be more uncertain than those obtained with other systems (cf. 197 Forbes et al. (2006) for a review). Finally, we did not consider nor include burnt 198 coarse woody debris, making the reasonable assumption that most PvC production in 199 savanna fires occurs close to the ground from grass, litter, and shrubs (Randerson et al., 2005). 200

201 A total of four burning experiments were conducted at each of the four study areas, with specific assessments of both initial biomass and PyC inventory carried out 202 before each burn. At a local scale the abundance and δ^{13} C values of litter and standing 203 biomass are quite heterogeneous in tropical savannas. This heterogeneity is largely 204 controlled by the distribution of trees, with lower average δ^{13} C values for litter and 205 206 soil C around trees compared to grass dominated areas away from the influence of 207 trees (Wynn et al., 2006; Saiz et al., 2012). Therefore, duplicate burns were conducted 208 at locations at half crown distance from trees ('Tree'; T locations), while two 209 additional burns were also conducted remote from trees ('Grass'; G locations). 210 Biomass was quantified in the near vicinity of each planned burn location by means of destructively collecting all aboveground plant material from two 1 m² census 211 212 quadrats. In order to estimate PyC lying on the soil surface, each quadrat was 213 subsequently vacuumed using a DC 23 Motorhead vacuum cleaner (Dyson 214 Appliances Ltd., NSW, Australia). This task was systematically performed by the 215 same user and over the same length of time across all burning experiments to allow 216 for inter-comparison of results. Both the biomass and the vacuumed material were 217 stored in separate labelled plastic bags. This sampling procedure was carried out in duplicate at each burning location, which resulted in a total of $8x1 \text{ m}^2$ census quadrats 218 219 being sampled per studied site (Fig. 3).

220 2.2.2 Experimental burns

Four small-scale (1 m^2) burning experiments were conducted at each of the four 221 222 study areas on sites with no recorded fires for two years during the late dry season of 223 2011. This period represents the maximum fuel load being available throughout the 224 year as a result of the presence of senesced grasses that have already reached their full 225 growth potential, and the fact that a number of tree species preferentially shed their 226 leaves at this time. All the burns took place on days with no prior recorded rainfall for 227 at least two weeks and shortly after midday, as this time generally corresponds to 228 maximum daily temperature (T). This combination of factors results in optimum 229 conditions for the occurrence and spread of fire in these ecosystems. At the time of the burns wind speeds were lower than 10 km h⁻¹ in all cases, air T ranged from 24.2 230 231 to 40.0 °C, and relative humidity varied from 45 to 17 %.

A rectangular area of approximately 3x2 m was consistently left undisturbed at 232 233 the centre of each planned fire. At this location, a purpose-built stainless steel 234 structure was deployed to allow for both the assessment and containment of the 235 experimental burning (Fig. 3). The final placement of the structure was chosen 236 considering both the prevailing wind and topography with a view to strategically allocate an untouched area of vegetation (>1 m^2) over which the fire could be ignited 237 238 promoting a natural progression of the flames towards the unit. Once the structure was 239 positioned on the ground, a 10 m radius buffer zone was cleared to the ground (Fig. 240 3).

241 The structure consisted of a series of leaning panels made up of lightweight 242 stainless steel, which was several times the volume occupied by the enclosed 243 vegetation. The leaning panels provided extra volume for an optimal collection of smoke and airborne particles, with their lower sides positioned at 15 cm above 244 245 ground-covering metal sheets to ensure a combustion with no artificial oxygen 246 limitation. The front panel of the unit was vertical and was open in its lower section to 247 allow the entrance of the fire. The structure was covered by an enclosed pyramid-like chamber culminating in an outlet to 15 cm diameter steel tube connected ultimately to 248 a Romac 2042K particle extraction unit (0.755 m³ s⁻¹, 2hp motor; Ron Mack, Perth, 249 250 WA, Australia). The extractor unit was in operation during the entire course of the 251 burn and was only disconnected after the cessation of any noticeable smouldering. A 252 125 µm stainless steel sieve was installed in the flow path at the outlet of chamber. 253 This mesh size was chosen to conform to standard dimensions used to differentiate 254 between micro- and macro-charcoal in palynological studies, e.g. Blackford (2000); 255 Haberle (2005). Smaller particles contained in the airflow downstream of the first 256 mesh were then subsampled and semi-quantitatively estimated using a particle 257 collector and a constant flow sampling pump (Quick Take 30, SKC Inc.,258 Pennsylvania, USA).

259 Continuous air flow monitoring was performed on a vertical pipe positioned 260 after the extractor unit by means of a calibrated 160S series 'S' type Pitot tube 261 (Dwyer Instruments, Inc., Indiana, USA) connected to a Testo 435-4 multi-function 262 instrument (Testo AG, Lenzkirch, Germany). Fire T was recorded by means of K-type 263 wire thermocouples connected to a data logger (Simple Logger II L642, AEMC 264 Instruments, USA) logging at 5 s intervals. The thermocouples were placed at the 265 centre of the burned area at 0.02 m above the ground, and at the outlet of the chamber. 266 Fire residence time was defined as the length of time that air T at the chimney's outlet 267 exceeded 100°C. This temperature threshold accounts for most of the particulate 268 emissions derived from a flaming front, and it is also likely to encompass the majority 269 of smouldering emissions in grass-dominated ecosystems.

270 The fire was allowed to burn the biomass contained within the unit without any 271 external intervention. After the flames self-extinguished, the leaning side panels of the 272 structure were lowered to the ground to minimise any lateral export of the burned material. Once the unit cooled down, any remaining stubble within the enclosed burnt 273 274 was cut at ground level, and the same vacuum procedure employed on the two 275 adjacent biomass quadrats was used again to determine total organic carbon (TOC) 276 and PyC remaining on the soil surface. In order to quantify and analyse the fine soot 277 produced during each burn, all the panels making up the structure were dismantled 278 and thoroughly cleaned by manually brushing with a water/methanol 1:1 solution, 279 with the resultant mixture being stored in a glass flask. Likewise, particulates 280 collected on the steel mesh and in the particle collector were then retrieved and carefully stored to subsequently weighed and analyzed as described in the laboratorymethods below.

In summary, each of the four experimental burns at each of four sites along the climate transect resulted in the field collection of the following samples:

- 285 2 biomass (vegetation quadrats)
- 3 ground-vacuumed (2 vegetation quadrats + 1 burnt quadrat)

287 - 3 airborne particulate samples from the burnt quadrat (1 from the steel sieve $288 > 125 \ \mu\text{m}, 1 \ \text{from the particle collector, and 1 from the soot adhered to the}$

289 metal panels).

- 290 2.3 Laboratory methods
- 291 **2.3.1 Initial preparations**

292 Biomass collected from each unburned reference quadrat was weighed, dried at 293 60°C for five days and re-weighed to determine fuel moisture. This material was then 294 sorted according to either grass (C_4) or woody biomass (*i.e.* tree/shrub leaves, twigs; 295 C_3). The vacuumed material from each quadrat was also sorted, and any biomass 296 fragments found were cleaned and added to the corresponding vegetation category 297 (*i.e.* grass or woody). Thereafter these were weighed, recombined (keeping some 298 aliquots separate for further testing) and milled. The samples vacuumed from the 299 quadrats (both pre-burn and post-burn) were sieved to 2 mm and weighed. Separate 300 aliquots were then size fractionated by wet sieving at 125 and 10 µm to conform with 301 the definition of microcharcoal (10-125 µm; Haberle 2005). This procedure enables 302 separate analysis of very fine $<10 \mu m$ PC that is likely to be a major component of 303 aerosol PC (Andreae and Merlet, 2001).

304 In the case of airborne particles captured in the system, the following 305 preparation procedures were conducted: (i) the coarse airborne particles collected at 306 the 125 µm steel sieve positioned at the chamber outlet were flushed with a water/ 307 methanol solution (1:1) and underwent the same wet fractionation procedure as 308 described above; (ii) the particles that passed through the steel mesh ($<125 \mu m$) were 309 wet-sieved to 10µm; and (iii) the soot retrieved after cleaning the structure with the 310 water/methanol solution was placed in a rotary evaporator to remove the liquid phase, 311 but no attempt was made to size separate this soot, as intense physical brushing may 312 have significantly altered the 'natural' particle size distribution. This fraction was 313 subsequently added to the distal (<125 μ m) pool. In all cases presented above, the 314 resultant fractions were subsequently freeze-dried, weighed and finely milled in 315 preparation for further analyses.

316 2.3.2 Hydrogen Pyrolysis

317 Hydrogen Pyrolysis has been described in detail in a number of publications (e.g., Ascough et al., 2009; Meredith et al., 2012; Wurster et al., 2012, Wurster et al., 318 2013). The technique separates PvC in aromatic clusters with a ring size >7 (HvPvC = 319 320 PyC that is likely to be resistant to environmental degradation) from other organic C 321 and has been shown to perform well in characterizing PyC abundance in a range of 322 environmental matrices (Meredith et al., 2012). Briefly, the solid samples were loaded 323 with a molvbdenum catalyst (~10% of dry weight) using an aqueous/methanol 324 solution of ammonium dioxydithiomolybdate [(NH₄)₂MoO₂S₂]. Dried, catalyst loaded samples were placed in a reactor and pressurized with 150 bar H₂ under a sweep gas 325 flow of 5 L min⁻¹, then heated at 300°C min⁻¹ to 250 °C, then stepped at 8°C min⁻¹ to 326 327 a final hold T of 550°C for 2 minutes.

Because the catalyst that is loaded undergoes weight loss during hydrogen pyrolysis, the abundance of carbon in the sample after hydrogen pyrolysis is determined relative to TOC (the mass of carbon after treatment / the mass of carbon loaded) and reported as %HyPyC/TOC or %HyPyC/Sample.

332 2.3.3 Carbon abundance and isotope composition

Carbon abundance and isotope composition of samples were determined using a Costech Elemental Analyzer (EA) fitted with a zero-blank auto-sampler coupled via a ConFloIV to a ThermoFinnigan DeltaV^{PLUS} mass spectrometer using Continuous-Flow Isotope Ratio Mass Spectrometry (EA-IRMS) at James Cook University's Cairns Analytical Unit. Stable isotope results are reported as per mil (‰) deviations from the VPDB reference standard scale for δ^{13} C values. Precisions (S.D.) on internal standards were better than ±0.2‰.

340 2.4 Calculations and Modelling

Measured quantities of TOC, PyC and HyPyC in each fraction were summed into two fluxes from each of the fires: "distal" and "proximal". 'Proximal' (likely to remain close to the site of burning) includes that collected from the surface after the fire in addition to the >125 μ m fraction collected from the sieve. 'Distal' (likely to be transported from the site of burning) includes the <125 μ m fraction collected from the stainless steel sieve, as well as that collected in the particle collector attached to the pump, and the soot cleaned from the apparatus.

348 The percent grass biomass at each site was estimated from the mass of separated 349 grass and woody biomass collected from two plots adjacent to the burning apparatus. 350 An estimate of the initial isotopic composition of the biomass ($\delta^{13}C_{biomass}$) was 351 determined by two methods. First a bulk sample of the biomass from the two vegetation plots was homogenized and its δ^{13} C value measured. Second, the 352 353 separately summed masses of grass and woody vegetation were used to estimate the 354 bulk isotopic composition, assuming values for C₃ woody biomass (-27.2‰) and for C₄ grass biomass (-13.1‰; values from Cerling et al., 1997). The estimated percent 355 grass biomass values produced by these two methods agreed (linear regression $r^2 =$ 356 357 0.88). The average of the two estimates is used as the central tendency while the range 358 of values is used as an error in these estimates. The carbon isotopic composition of CO₂ and other trace gases produced by combustion for each of the fires was 359 360 calculated by mass balance using the amount and isotopic composition of initial 361 biomass and of the residual products of combustion (note that this mass difference 362 calculation of CO₂ likely includes other carbonaceous trace gases such as CO and 363 CH₄).

364 The value of SIDE can be expressed as an 'isodisequibrium forcing coefficient' 365 following the terminology of Alden et al. (2010), which is a difference between the δ^{13} C values of forward and reverse fluxes between two reservoirs. The values of SIDE 366 367 were calculated for each of the component fluxes from the fires as the difference between the δ^{13} C value of the flux (δ^{13} C_{flux} for example the distal HyPyC component, 368 $\delta^{13}C_{\text{flux}} = \delta^{13}C_{\text{distal HyPvC}}$ and the initial $\delta^{13}C$ value of the biomass ($\delta^{13}C_{\text{biomass}}$). The 369 370 values of SIDE from each of the four fluxes was regressed with respect to the estimate 371 of percent grass biomass using the Matlab curve fitting toolbox with a power function. 372 Significance of the power law curve fit was calculated as a *p*-value, which describes 373 the probability with an F-test that the relationship is a better estimate than simply the 374 mean value of SIDE. Confidence intervals for the curve fit equations were calculated at p = 0.9. The distribution of residuals were tested for normality with a Jarque-Bera test.

377 The savanna isotopic disequilibrium flux ($D_{SIDE CO2} = F_{bur}(SIDE_{CO2})$, where F_{bur} 378 is the flux of C from annual biomass burning) was calculated for global savanna 379 environments using an estimate of the fraction of C₄ photosynthesis from Still et al. 380 (2003). The calculated F_{bur} value for savannas was averaged over the period from 381 1997-2011 using the Global Fire Emissions Database (GFED; 382 http://globalfiredata.org; van der Werf et al., 2010), clipped to the area where C₄ 383 grasses > 1% (Still et al., 2003). The global distribution of the isotopic disequilibrium 384 flux was calculated and plotted using Generic Mapping Tools (GMT; Wessel et al., 385 2013).

386 **3 Results**

387 3.1 Production of PyC during savanna burning

388 The total carbon exposed (TCE) to combustion showed a discernable pattern across the precipitation gradient, with higher potentially combustible C in standing 389 390 biomass and surface litter at the more humid sites (Table 2, Fig. 2). The median 391 [range] production of PyC and HyPyC was 16.0 [11.5]% and 2.5 [4.9]% of TCE 392 across all experimental burns (Table 2). Similarly, the amount of total carbon 393 combusted (TCC) defined as the difference between TCE and post fire carbon was 394 used to calculate combustion completeness (described as TCC relative to TCE), and 395 whose median [range] for all fires was 84.0 [11.5)%. The median [range] amount of 396 HyPyC in the proximal component (>125µm) of the flux from the fires was 96.9 397 [14.1]% of the total HyPyC flux across all experimental burns. Fire residence time, 398 defined as time over which air T at the chimney's outlet exceeded 100°C, increased

with TCE (Fig. 4), and thus with the proportion of woody biomass (Table 2). The
proportion of TCE converted to HyPyC significantly decreased with initial TCE, and
thus decreased with the proportion of woody biomass (Fig. 4; Table 2).

402 **3.2** Isotopic disequilibria of proximal and distal fluxes of PyC and HyPyC

The values of SIDE were computed from δ^{13} C values shown in Table 3. When 403 considering the PyC pool, the median [range] SIDE value of proximal PyC 404 (SIDE_{proximal-PvC} -3.3 [4.2]‰; where SIDE = $\delta^{13}C_{\text{flux}}$ - $\delta^{13}C_{\text{biomass}}$, with superscripts 405 406 proximal or distal and PyC or HyPyC are relevant to the flux) was not significantly different from the median [range] SIDE value of distal PyC (SIDE_{distal-PyC}; -4.7 407 408 [6.3]%; p = 0.737, Mann-Whitney U; Fig. 5). However, the median [range] SIDE 409 value of proximal HyPyC (SIDE_{proximal-HyPyC} -1.1 [5.3]‰ was significantly different from the median [range] SIDE value of distal HyPyC (SIDE_{distal-HyPyC}; -2.9 [8.0]‰; p 410 411 = 0.024; Fig. 5). Comparing the two proximal fluxes, the median value of $SIDE_{proximal}$ _{PvC} was significantly different from the median value of SIDE_{proximal-HvPvC} (p < 0.001412 413 Fig. 5). The median value of SIDE_{distal-PyC} was not significantly different from the 414 median value of $SIDE_{distal-HvPvC}$ (p = 0.245; Fig. 5). The median [range] SIDE value of 415 CO_2 of combustion (SIDE_{CO2}) was 0.61‰ [0.88‰] (Fig. 5).

Neither SIDE_{proximal-PyC}, nor SIDE_{proximal-HyPyC} show a significant relationship to the initial proportion of C₄ biomass (p = 0.50; and p = 0.44, respectively; Fig. 6). However, SIDE_{distal-PyC} shows a significant power-law relationship to the initial proportion of C₄ biomass (SIDE_{distal-PyC} = -1.068 x 10⁻⁵ (%grass)^{2.862} – 0.661; R² = 0.789; p = 0.007), with values decreasing towards the C₄-dominated end of the vegetation gradient (Fig. 6). Similarly, SIDE_{distal-HyPyC} was positively correlated with the initial proportion of C₄ biomass (SIDE_{distal-HyPyC} = -3.243 x 10⁻⁷ (%grass)^{3.589} – 423 0.298; $R^2 = 0.577$; p = 0.094). SIDE_{combustion-CO2} showed no significant relationship to 424 the initial proportion of C₄ biomass (p = 0.99).

425 4 Discussion

426 4.1 Production of PyC across contrasting savanna environments

427 The proportion of biomass converted to PyC and HyPyC during the experimental burns varied across the range of savannas studied (Table 2, Fig. 4). The 428 429 median PyC/ TCE conversion rate of 16.0% is in the upper range of values reported in 430 previous studies, although those could have underestimated actual PvC conversion 431 rates due to a combination of factors, which include the use of analytical techniques 432 focusing only on recalcitrant PyC components, the non-inclusion of all PyC materials 433 being produced, and the choice of experimental fires not being representative of 434 typical wildfire conditions (Santín et al., in press). However, the observation that the 435 relative amount of HyPyC produced in grass-dominated savannas was larger than in 436 woody-biomass-dominated savannas runs counter to our initial assumption that 437 anticipated that more woody biomass would promote the production of proportionally 438 more HyPyC. Longer fire residence times were positively correlated with greater TCE, 439 which resulted in less HyPyC being formed due to the opportunity for more complete 440 combustion (Fig. 4). Indeed, incomplete combustion of biomass can mainly be 441 ascribed to the combined effects of low combustion T, short fire residence times, and 442 an overall lack of available oxygen (Loo and Koppejan, 2002).

Typically, low fire residence time is reported for fast moving fires characteristic
of landscapes with abundant fine fuel, such as savannas (Hartford and Frandsen,
1992; Wright and Bailey, 1982). While fire residence time was shorter for the grassdominated sites, maximum T was quite comparable across all the studied sites (Table

2), and agrees well with results reported for savanna fires elsewhere (Miranda et al.,
1993; Trollope, 1984). Therefore, the short-lived fires occurring in grass-dominated
ecosystems may result in the production of proportionally more HyPyC (Table 2, Fig.
4). The median [range] production of HyPyC as a proportion of both TCE and TCC
were 2.5 [4.9]% and 2.9 [5.9]% respectively. These figures compare well with
estimates of 'black carbon' (charcoal) production for savanna and grassland fires
reviewed by Forbes et al. (2006).

454 4.2 Allocation of HyPyC produced during savanna fires

455 In order to better constrain the global PyC cycle, there is an obvious need for a 456 deeper understanding of the factors controlling formation, translocation and 457 mineralisation of PyC and its recalcitrant compounds, represented here by HyPyC 458 (Bird et al., 2015; Conedera et al., 2009; Zimmermann et al., 2012). In this context, 459 the partitioning of PyC between the proximal and distal fluxes, may have a strong 460 influence on preservation potential (Thevenon et al., 2010). Blackford (2000) reports 461 an order of magnitude decrease in the percentage of charred particles >125 µm 462 observed at just 7 m from a recently burned area. In contrast, smaller size particles 463 can be transported by wind up to thousands of kilometres, with this range largely 464 depending on the particle size, height of the convection column and environmental 465 conditions (Clark, 1988). The total amount of HyPyC contained in both fluxes varied 466 across the burning experiments, but with most of the HyPyC in the proximal flux in 467 all cases. The proportion of HyPyC in the proximal flux was very large in grass-468 dominated savannas (>96% of total HyPyC at MIT), compared to the proportion of 469 HyPyC in the proximal flux in woody-biomass-dominated savannas (86-91% of total 470 HyPyC at DCR). This also suggests that longer fire residence times not only result in 471 a more complete combustion of biomass, they may also promote more effective 472 comminution and volatilisation of fuel into fine particles and gases. Such an effect
473 was also observed by Kuhlbusch et al. (1996) who found burning experiments with
474 the highest fuel loads showed the highest degree of C volatilisation.

475 **4.3 Savanna isotope disequilibrium effects (SIDE)**

In general, the calculated SIDE values are consistent with relative ¹³C-depletion 476 477 of PyC produced during savanna fires (Krull et al., 2003), with some significant 478 distinctions between proximal and distal fluxes, as well as between the PyC and the HyPyC fluxes (Fig. 6). A relative ¹³C-depletion is more consistently observed for the 479 proximal PyC, with δ^{13} C values always lower than the original biomass. The δ^{13} C 480 481 values of the proximal flux of HyPy C were also generally lower, with the exception of two fires (UND 3-4; Table 3, Fig. 5). The distal flux of PvC also had lower δ^{13} C 482 values than the precursor biomass. The observation that PyC is relatively ¹³C-depleted 483 484 with respect to the original biomass across all sites, supports the hypothesis that, 485 where woody (C_3) and grass (C_4) biomass are both present, the (C_4) grass biomass is 486 preferentially combusted relative to (C_3) woody material, leaving PyC from woody 487 biomass to contribute disproportionately to the PyC flux (i.e., SIDE_{PyC} values are negative). Also because the δ^{13} C values of PyC are relatively 13 C-depleted with 488 respect to original biomass, mass balance dictates that the δ^{13} C values of CO₂ of 489 combustion are relatively ¹³C-enriched with respect to original biomass (*i.e.*, SIDE_{CO2}) 490 491 values are positive Fig. 5).

This differential fractionation between the products of combustion and original biomass may represent a significant contribution to the global carbon isotope disequilbrium terms used in ¹³C double deconvolution models of atmospheric CO_2 (Alden et al., 2010; Ciais et al., 2005; Enting et al., 1995). The isodisequilibrium 496 forcing coefficient for biomass burning, here referred to as I_{bur} , is defined as the 497 difference in the d¹³C value of CO₂ produced by biomass burning and the d¹³C value 498 of CO₂ into biomass regrown after burning:

499
$$I_{bur} = (\delta^{13}C_{CO2_bur} - \delta^{13}C_{CO2_regrow_bur})$$
(1)

500 while the disequilibrium associated with this term (D_{bur} of Ciais et al. (2005) is:

$$501 D_{bur} = F_{bur}(I_{bur}) (2)$$

502 where F_{bur} is the atmospheric source flux of CO_2 due to biomass burning.

However, as used in double deconvolution mass balance models, I_{bur} assumes no isotopic fractionation associated with combustion, *i.e.*, that the $\delta^{13}C$ value of CO₂ of combustion ($\delta^{13}C_{CO2_bur}$) is equal to that of biomass burnt ($\delta^{13}C_{biomass_bur}$). Because our results show that CO₂ of combustion is ¹³C-enriched with respect to biomass burnt (by a SIDE_{CO2} value of about 0.6‰), we can explicitly account for this during the calculation of I_{bur} :

509
$$I_{bur*} = (\delta^{13}C_{biomass_bur} + SIDE_{CO2} - \delta^{13}C_{CO2_regrow_bur})$$
(3)

An asterisk is used to indicate that the term accounts for fractionation duringcombustion. The total burning disequilibrium becomes:

512
$$D_{bur*} = F_{bur} \left(\delta^{13} C_{biomass_bur} - \delta^{13} C_{biomass_regrow_bur} \right) + F_{bur} (SIDE_{CO2})$$
(4)

513
$$D_{bur*} = D_{bur} + D_{SIDE_CO2}$$
(5)

The first term is the disequilibrium as calculated by Ciais et al. (2005), and assumes no fractionation associated with combustion. The second term explicitly accounts for SIDE_{CO2} associated with differential combustion of C₃ and C₄ biomass. The global value of D_{SIDE_CO2} is estimated in Figure 7, to have a global sum of about 0.75 Gt C 518 yr^{-1} ‰. This value may be an upper bound for D_{SIDE} because some of the ¹³C-depleted 519 PyC may be mineralized after the fire, as was observed by Zimmermann et al. (2012).

Figure 5 shows that in both the proximal and distal fluxes, the significance of SIDE during burning is more pronounced when considering the PyC produced by the fires than when considering the HyPyC component alone. The observed patterns highlight the importance of SIDE for the more labile or semi-labile components of the PyC flux, into which C₄-derived PyC may be preferentially partitioned. Whereas the significance of SIDE is less pronounced in the more stable pool of PyC flux, into which C₃-derived PyC may be preferentially partitioned.

527 We used particle size separations of PyC fluxes to test the hypothesis that PyC 528 remaining near to the site of production shows a more pronounced SIDE due to 529 preferential export of C₄-derived PvC in the finer particle size fractions. While no 530 significant trends are observed for the proximal fluxes of PyC (neither the total, nor 531 HyPyC components), the relationship is significant for both distal PyC and for distal 532 HyPyC (Fig. 6). These observations indicate that in grass-dominated environments, 533 the preferential export of fine particle size fractions is likely to be dominated by low 534 δ^{13} C components of PyC. Thus, the SIDE is more pronounced in grass-dominated 535 fires, either because (a) the small amount of C₃-derived PyC flux in these systems is 536 preferentially exported in the fine (soot) fraction of the distal component, or (b) compounds with low δ^{13} C values are preferentially exported to the fine fraction (Das 537 538 et al., 2010; Krull et al., 2003). Krull et al. (2003) attributed this effect to the occlusion of protected ¹³C-depleted compounds in phytoliths of C₄ grasses, and our 539 results further suggest that ¹³C-depleted compounds (*e.g.*, lipids, lignins or phytoliths) 540 541 may be preserved in PyC (O'Malley et al., 1997), being preferentially exported as

542 highly condensed aromatic structures in the fine soot components of natural fires. 543 Another significant contributor to SIDE may also be the plant methoxyl pool, which is extremely depleted in ¹³C (~-29‰ in leaves relative to bulk biomass) and comprises 544 about 2.5% of plant biomass (Keppler et al., 2004). Moreover, the same authors report 545 546 that the fractionation associated with the methoxyl-groups may be further enhanced 547 during their conversion to volatile compounds (i.e. through burning), which could also contribute to the low δ^{13} C values we observed in the soot fraction. Nonetheless, we 548 549 cannot rule out the possibility that a significant proportion of this 'soot' component 550 may derive from the small amount of C3 biomass present, even in C4 dominated ecosystems. 551

The small-scale experimental set-up used here did not consider coarse woody debris (CWD), which is common in most savanna environments and may burn along with other biomass on the ground. As this material is exclusively composed of C₃ biomass, the inclusion of PyC from CWD would likely increase the contribution of proximal PyC to the total flux, further enhancing the SIDE on SOC δ^{13} C (Bird and Pousai, 1997; Wynn and Bird, 2008).

558 4.4 Implications of SIDE

The calculation of the global SIDE 'disequilibrium flux' (Fig. 7) reinforces the conclusion that the effects of SIDE during savanna burning are most pronounced in grass-dominated savannas, where C₄ plants are the most significant fraction of biomass. Scaling these trends up to the global scale indicates a significant SIDE forcing for the savanna biome, which may contribute to a ¹³C disequilibrium flux on the order of 0.75 Gt C yr⁻¹ ‰, a value which is of significant magnitude in comparison to other ¹³C disequilibrium fluxes used in mass balance models (Ciais et al., 2005). This ¹³C-disequilibrium flux (D_{SIDE_CO2}) accounts for isotope fractionation associated with differential combustion of C₃ and C₄ biomass, which is otherwise not explicitly accounted for, and would contribute to the total biomass burning isotopic disequilibrium (D_{bur}) caused by regrowth of ecosystems after fire (1.66 Gt C yr⁻¹ ‰; Ciais et al.; 2005).

571 Because grass-dominated savannas show high SIDE for PyC, the implication is 572 that SOC in savannas with frequent grass-dominated fires will become increasingly ¹³C-depleted, as the stable components of PyC accumulate in the soil over time. This 573 574 observation is consistent with studies of SOC at large spatial scales; for example where grass-dominated Mitchell grasslands show lower δ^{13} C values of surface SOC 575 (ca. -16‰) than is typical of C₄ biomass (ca. -12‰) (Bird and Pousai, 1997; Wynn 576 577 and Bird, 2008), and agrees well with findings by Dümig et al. (2013) which indicate both the presence of charred grass residues and accumulation of alkyl C in soil 578 fractions as the most likely contributors to the observed decreasing δ^{13} C values from 579 grass biomass to C₄-derived surface SOC. Such a conclusion has implications for 580 581 interpretation of paleorecords derived from ancient SOC or its by-products in sedimentary records, which may be biased towards ¹³C-depleted values by this SIDE 582 583 during savanna burning.

584

585 **5 Conclusions**

The production of PyC and HyPyC was quantified in sixteen experimental fires conducted along a transect of sites in northern Australian savannas. The residues after burning were partitioned into proximal and distal fluxes, each measured for their PyC and HyPyC contents and the carbon isotope composition of each component. The 590 production of PyC across all experimental burns ranges from 11.6 to 23.1% of TCE. 591 TCE is positively correlated with fire residence time, and increasing fire residence 592 time reduces the proportion of HyPyC produced due to the opportunity for more 593 complete combustion. Thus, the short-lived fires occurring in grass-dominated 594 ecosystems result in the production of proportionally more HyPyC compared to 595 woodier-dominated savannas. The median [range] production of HyPyC as a 596 proportion of both TCE and TCC across all experimental burns were 2.5 [4.9]% and 597 2.9 [5.9]% respectively. These figures are in good agreement with other estimates of 598 'black carbon' (charcoal) production for savanna and grassland fires (Forbes et al., 599 2006). The relative amount of HyPyC in the proximal flux is significantly larger in 600 grass-dominated savannas (up to >99%) compared to that observed in woodier 601 savannas (>86%). This is a significant observation as these two fluxes are likely to 602 have different preservation potentials. The Savanna Isotope Disequilibrium Effect 603 (SIDE, difference in carbon isotopic composition between grass biomass and 604 pyrogenic carbon components) was highest at sites with the greatest proportion of 605 grass biomass although this trend was only observed for the distal flux of fine-grained 606 material (<125µm). Scaling the SIDE values observed here to the global savanna biome suggests that the SIDE effect contributes a significant ¹³C-disequilibrium flux 607 608 to the global mass balance of ¹³C exchange between the atmosphere and other 609 reservoirs (Ciais et al., 2005). The observation that SIDE increases with increasing 610 grass biomass also suggests that frequently burned savannas are subject to depletion of ¹³C in the pyrogenic products. As the PyC produced by savanna fires accumulates, 611 612 either in situ, or distally, the SIDE effect produces soil or sedimentary organic carbon with δ^{13} C values which are relatively 13 C-depleted with respect to the original 613 614 biomass. This effect may have significant implications for the interpretation of stable 615 carbon isotopic composition of paleorecords which are based wholly or in part on616 PyC.

617 Author contribution

GS, JW, IG and MB designed the experiment. GS and IG carried out the fieldwork and conducted laboratory analyses. JW developed the model and performed simulations. GS, JW and MB prepared the manuscript with contributions from all co-authors.

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Table 1: Characteristics of the studied sites.

Site	Latitude	Longitude (°F)	MAP (mm)	MAT (°C)	CC
DCR (Davies Creek NP)	16.997	145.574	2050	21.3	55
BRK (Brooklyn Nature Refuge)	16.586	145.155	1650	22.5	40
UND (Undara NP)	18.208	144.658	795	23.6	30
MIT (Mitchell Grassland)	21.403	144.677	435	24.3	5

MAP = mean annual precipitation and MAT = mean annual temperature. Climate data derives from the Australian Bureau of Meteorology. For the calculation of woody

canopy cover (CC) the reader is referred to Domingues et al. (2010).

HyPyC/] TCE (%)	proximal/ Post-fire C total combustion, HyPyC balance cale
5	98 220.0
З	99 285.9
6	99 130.6
4	96 175.3
4	100 319.6
4	97 311.0
1	97 267.1
¹ na	¹ na ¹ na
2	94 346.5
2	97 408.5
4	97 293.5
2	96 421.1
² na	² na ² na
1	91 695.4
1	85 382.2
1	90 405.1
2.5	96.9 333.0
burned ard	
$\begin{array}{c c} (\%) \\ 5 \\ 6 \\ -1 \\ 1 \\ -1 \\ -1 \\ -2 \\ -2 \\ -2 \\ -2 \\ -2 \\ -2 \\ -2 \\ -2$	

Notes: ¹Mass balance in this experimental burn was not possible as the extraction pipe accidentally detached from the structure during the fire. ²Sample was contaminated during manipulation and storage.

DCK4(0) -21.2		DCR 3 (G) -17.8	DCR 2 (T) -22.2	DCR 1 (T) -22.2	BRK 4 (G) -14.1	BRK 3 (G) -13.1	BRK 2 (T) -16.3	BRK 1 (T) -14.8	UND 4 (T) -15.7	UND 3 (T) -18.9	UND 2 (G) -15.1	UND 1 (G) -14.4	MIT 4 (T) -14.7	MIT 3 (T) -14.4	MIT 2 (G) -14.3	MIT 1 (G) -14.5	δ ¹³ C	FIFE EXP. TOC	rin biom	pre-fi	
(9.1) -15.5 (9.1)	-21.1	-20.4	-22.3	-21.4	-15.0	-13.9	-16.7	-15.7	-17.6	-19.2	-15.3	-14.8	-13.5	-13.3	-13.2	-13.2	(‰) $\delta^{13}C$ (‰)	mass-based	ass biomass	ire ¹ pre-fire	
-20.0 (9.0)	-25.6	-25.7	-25.9	-25.4	-21.5	-18.0	-18.0	-21.9	-23.9	-23.1	-19.1	-18.9	-18.1	-16.9	-16.6	-16.7	(‰)	TOC δ^{13} C	ground	pre-fire	
-16.5 (8.7)	-22.7	-20.4	-23.1	-22.8	-16.3	-14.4	-16.6	-16.8	-18.4	-20.2	-16.2	-15.7	-15.1	-14.7	-14.7	-14.8	δ ¹³ C (‰)	TCE	measured	² pre-fire	
-19.0 (11.5)	-25.4	-24.7	-25.4	-24.3	-21.9	-20.0	-22.3	-21.2	-17.9	-17.9	-15.9	-15.8	-16.2	-15.8	-15.2	-13.9	$\delta^{13}C$ (‰)	HyPyC	ground	pre-fire	
-20.7 (9.7)	-25.2	-25.6	-25.7	-25.7	-20.7	-20.3	-22.6	-20.7	-23.2	-21.6	-17.8	-18.7	-17.3	-17.0	-16.7	-16.0	(‰)	$PyC \delta^{13}C$	proximal	post-fire	
-17.2 (9.1)	-22.4	-23.9	-22.9	-23.0	-16.8	-16.4	-18.5	-16.5	-17.7	-17.9	-16.7	-17.5	-15.5	-15.5	-16.4	-14.8	δ ¹³ C (‰)	HyPyC	proximal	post-fire	
-20.6 (5.8)	-22.4	-23.4	-23.3	⁴ na	-20.6	-18.8	-21.3	-20.0	-20.2	-20.8	-17.6	-20.8	-19.7	-20.5	-20.4	-20.7	post-fire distal PyC δ ¹³ C (‰)		post fire		
-19.6 (7.6)	-22.1	-23.1	-23.5	⁴ na	-19.8	-18.8	-19.8	-19.1	-18.8	-17.9	-16.9	-19.6	-19.6	-20.1	-20.1	-15.9	post-fire distε HyPyC δ ¹³ C (‰)				
-15.7 (9.6)	-22.0	-19.3	-22.8	⁴ na	-15.6	-13.2	-15.7	-16.2	³ na	-19.9	-15.8	-15.2	-14.8	-14.2	-14.3	-14.4	calculated (‰)	mass-balance	¹¹ of combustion,	Post-fire CO_2	

Explanations for the different fractions are as in Table 2 **Table 3**: Stable isotopic composition (δ^{13} C values) of C fluxes from each of the fires; standard errors of the means are shown in parentheses.

and for C_4 grass biomass (-13.1%); values from Cerling et al. (1997). Notes: estimated carbon isotopic composition based on the mass of woody and grass biomass, assuming values for C_3 woody biomass (-27.2 %)

 2 TCE = total carbon exposed, mass-weighted isotopic composition of pre-fire biomass and ground TOC.

was contaminated during manipulation and storage. ³Mass balance in this experimental burn was not possible as the extraction pipe accidentally detached from the structure during the fire. ⁴Sample

Figures



Figure 1: Geographical locations of the studied sites in northeast Australia. Source: "North East Australia" Image Landsat (2014) Google Earth.



Figure 2: Characteristics and regional classification of the vegetation; the reader is referred to Torello-Raventos et al. (2013).



Figure 3: Schematic diagram depicting an experimental burn, with pre- and post-fire C pools. Initial biomass and PyC inventory were quantified in the near vicinity of each planned burn location by means of destructively collecting all above-ground plant material and vacuuming the soil surface from two 1m² census quadrats, as shown in left bottom picture.



Figure 4: (a) Relationship between total carbon exposed (TCE) and fire residence time. The regression (dashed line) takes the following form: *Minutes over 100 °C* $= 8 \times 10^{-5} (TCE)^{1.719}$; $r^2 \ 0.66$; p < 0.05; *BIC (Bayesian Information Criterion)* 3.47; n=14. (b) Relationship between TCE and the proportion of pyrogenic carbon produced (HyPyC) relative to TCE. The regression (solid black line) takes the following form: *HyPyC/TCE (%)* = $48.6 - 30.9 (TCE)^{0.066}$; $r^2 \ 0.61$; p < 0.05; *BIC* 6.27; n=14. Different symbols group the individual burning experiments into the different ecosystems shown in Figure 2; individual data is presented in Table 2.



Figure 5: Box and whisker plot of isotopic disequilibrium values (SIDE = $\delta^{13}C_{\text{flux}}$ - $\delta^{13}C_{\text{pool}}$) for proximal and distal fluxes of PyC and HyPyC, and combustion CO₂ calculated by mass-balance (n=16 for proximal components; n= 15 for distal components, n=14 for combustion CO₂). Line at centre of box represents the sample median, box represents 25th and 75th percentiles, and whiskers represent range.



Figure 6: Relationship of isotopic disequilibrium values (SIDE = $\delta^{13}C_{flux}-\delta^{13}C_{pool}$) to the initial proportion of grass biomass (%, by mass). Error bars of % grass biomass represent the range of values measured from two 1 m² quadrats. Error bars of $\Delta \delta^{13}$ C represent values calculated using the difference from the isotopic composition of standing biomass ($\delta^{13}C_{pool}$) as determined by one of two methods: (1) measurement of the bulk C isotopic composition of biomass collected from the two quadrats, and (2) estimated carbon isotopic composition based on the mass of woody and grass biomass, assuming values for C₃ woody biomass (-27.2 ‰) and for C₄ grass biomass (-13.1‰; values from Cerling et al., 1997). Solid line shows best curve fit of power-law equation, with dashed lines representing 90% confidence intervals. n=16 for proximal components; n= 15 for distal components. Residuals of the power law fits to the distal HyPyC and distal PyC data showed a normal distribution (Jarque-Bera), with mean residual SIDE values of -0.03 and-0.02‰.



Figure 7: Global map of savanna isotope 'disequilibrium flux' (following terminology of Alden et al. (2010) $D_{SIDE_CO2} = SIDE_{CO2} \times F_{bur}$) of the flux of CO₂ of combustion. The value of SIDE_{CO2} used is 0.61‰. F_{bur-savanna} is the mean annual flux of CO₂ from savanna fires, averaged over the period from 1997-2011 using the Global Fire Emissions Database (GFED; http://globalfiredata.org; van der Werf et al., 2010), clipped to the area where C₄ grasses > 1% (Still et al., 2003), with an estimated fraction of combusted CO₂ of 0.83 (estimated from Table 2).