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Article type : Articles

Running title: Land transformation: C3, C4 decomposition

**Land transformation in tropical savannas preferentially
decomposes newly added biomass, whether C3- or C4-derived**

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Manuscript received 9 October 2019; revised 20 February 2020; accepted 15 April 2020.

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the [Version of Record](#). Please cite this article as [doi: 10.1002/EAP.2192](https://doi.org/10.1002/EAP.2192)

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Abstract

As tropical savannas are undergoing rapid conversion to other land uses, native C3-C4 vegetation mixtures are often transformed to C3- or C4-dominant systems, resulting in poorly understood changes to the soil carbon (C) cycle. Conventional models of the soil C cycle are based on assumptions that more labile components of the heterogeneous soil organic C (SOC) pool decompose at faster rates. Meanwhile, previous work has suggested that the C4-derived component of SOC is more labile than C3-derived SOC. Here we report on long-term (18 month) soil incubations from native and transformed tropical savannas of northern Australia. We test the hypothesis that, regardless of the type of land conversion, the C4 component of SOC will be preferentially decomposed. We measured changes in the SOC and pyrogenic carbon (PyC) pools, as well as the carbon isotope composition of SOC, PyC and respired CO₂, from 63 soil cores collected intact from different land use change scenarios. Our results show that land use change had no consistent effect on the size of the SOC pool, but strong effects on SOC decomposition rates, with slower decomposition rates at C4-invaded sites. While we confirm that native savanna soils preferentially decomposed C4-derived SOC, we also show that transformed savanna soils preferentially decomposed the newly added pool of labile SOC, regardless of whether it was C4-derived (grass) or C3-derived (forestry) biomass. Furthermore, we provide evidence that in these fire-prone landscapes, the nature of the PyC pool can shed light on past vegetation composition: while the PyC pool in C4-dominant sites was mainly derived from C3 biomass, PyC in C3-dominant sites and native savannas was mainly derived from C4 biomass. We develop a framework to systematically assess the effects of recent land use change versus prior vegetation composition.

Keywords: soil carbon, pyrogenic carbon, decomposition, tree:grass ratio, C3:C4, savanna

1 Introduction

Although it is widely recognized that land cover–land use change (LCLUC) may have significant impacts on the global soil organic carbon (SOC) pool, changes in the size, structure and stability of the SOC pool resulting from LCLUC remain among the most uncertain components of the global carbon (C) cycle [Post and Kwon, 2000; Hopkins *et al.*, 2012; Liu *et al.*, 2015; van Groenigen *et al.*, 2017; Bond-Lamberty *et al.*, 2018; Le Quéré *et al.*, 2018]. Recent C accounting and global modelling efforts have identified tropical semi-arid ecosystems as a largely climate-driven C sink exhibiting high inter-annual variability [Poulter *et al.*, 2014; Liu *et al.*, 2015], highlighting the significance of tropical savannas to the global C cycle. While these results demonstrate the dynamic nature and global significance of the tropical savanna C cycle, they also highlight current limitations in our understanding of this C cycle, in particular the residence time of accumulating SOC, and how this can be influenced by climate and land management.

It is currently difficult to predict how shifting patterns and properties of vegetation resulting from LCLUC drive changes in the SOC pool. Future progress in developing quantitative model predictions will require new methods to establish relationships between more readily quantifiable changes in vegetation composition and structure and the resultant changes in the chemical properties of plant biomass and litter which ultimately control changes in the SOC pool [Martens, 2000; Cotrufo *et al.*, 2013; Tamura and Tharayil, 2014]. Thus, more robust projections of how savanna ecosystems act as long-term C sources or sinks will continue to depend on the development of novel methodologies to quantify the response of the SOC pool to different LCLUC scenarios.

The tropical savannas of northern Australia occupy 1.93 million km² [Fisher and Edwards, 2015] – a quarter of the Australian land mass – and account for ~10% of the world's savanna [Hutley and Setterfield, 2019]. LCLUC in this region is driven by grazing of native grass savannas, clearing for improved pasture, cropping, mining and plantation forestry. Landsberg *et al.* [2011] estimated that by 2010, 92,000 km² of tropical savanna had been cleared since European settlement, mostly in Queensland with clearing across this region ongoing at ~790 km² y⁻¹ [Bristow *et al.* 2016]. In high rainfall areas (> 900 mm) of northern Australian savannas, vegetation cover change is also being driven by the proliferation of high-productivity, invasive grasses. These grasses can increase fuel load by a factor of five or more and trigger high severity

fires that limit the regeneration of native woody species [Rossiter *et al.*, 2003; Brooks *et al.*, 2010; Setterfield *et al.*, 2010; Levick *et al.*, 2015]. In addition, ‘undisturbed’ native savannas are subject to relatively high-frequency fire regimes, with return intervals commonly between 1-5 years depending on annual rainfall, fuel production and management practices [Yates *et al.*, 2008]. Fire regime has a significant impact on tree:grass ratios, with frequent, high intensity fire limiting woody persistence [Rossiter *et al.*, 2003; Lehmann *et al.*, 2014]. In such fire regimes, pyrogenic carbon (PyC), a product of biomass burning that is resistant to degradation [Bird *et al.*, 2015], is a key component of the belowground C pool [Reisser *et al.*, 2016; Santin *et al.*, 2016; Surawski *et al.*, 2016], particularly in savanna ecosystems [Forbes *et al.*, 2006; van der Werf *et al.*, 2010; Saiz *et al.*, 2015]. Although PyC is known to be a significant fraction of total C in Australian savanna soils [Qi *et al.*, 2017], there is a need to better understand factors controlling its distribution at the continental and global scales [Santin *et al.*, 2016].

Changes in vegetation structure and fire regime can both be expected to change the input of PyC to the soil, and potentially also the resistance of PyC to mineralization [Bird *et al.*, 2015]. Thus, weed invasion, altered fire regimes and/or LCLUC can result in monocultures of either trees or grasses, with poorly known ramifications for belowground C pools. While changes in the above-ground biomass of these altered savanna systems are relatively well quantified using robust, standard methods [Cook *et al.*, 2005; Williams *et al.*, 2005; Downie *et al.*, 2014], it has been more difficult to quantify short and long-term changes in the belowground SOC pool and pool structure [Bispo *et al.*, 2017]. Substantial knowledge gaps remain regarding the resilience and residence time of SOC under scenarios of LCLUC. Quantifying the response of both SOC and PyC to a range of LCLUC scenarios is therefore a pre-requisite to being able to predict impacts on C cycling in these important tropical savanna ecosystems.

In this study, we examine how rapid, well-quantified changes in observed LCLUC impacts on biomass structure may translate into similarly rapid changes in turnover rates of C3- and C4-derived C in the SOC and PyC pools. We use stable C isotope approaches that exploit the contrasting biogeochemical and isotopic signatures of these lifeforms in savanna and trace C movement. Such methodologies provide a unique opportunity to utilize the isotopic differences between tree (C3) and grass (C4) biomass in tropical savannas to examine how changes in tree:grass ratios propagate into secondary changes in the stability of SOC [Balesdent *et al.*, 1987;

Veldkamp, 1994; Krull *et al.*, 2005; Wynn and Bird, 2007; Wittmer *et al.*, 2010; Blagodatskaya *et al.*, 2011; Creamer *et al.*, 2011; Don *et al.*, 2011; Guillaume *et al.*, 2015; Zhang *et al.*, 2015].

Specifically, our study evaluates a range of native and transformed savanna sites where land use changes have resulted in almost exclusively C3 and C4 biomass. We re-examine systematic differences in the decomposition rate of SOC derived from the C3- and C4-portions of mixed systems as in Wynn and Bird [2007], and extend this approach to address the following questions regarding LCLUC in tropical savannas: (1) How do different LCLUC scenarios affect the SOC, PyC pools and their turnover rates? (2) Is the preferential decomposition of C4-derived biomass consistently observed across a variety of LCLUC scenarios? (3) Does the relative proportion of C4-derived biomass in the PyC pool vary with different LCLUC scenarios, and can these variations be related to past vegetation history?

2 Methods

2.1 Site descriptions

We took replicated, shallow soil cores (0-30 cm) from seven sampling sites that span a range of land uses (Table 1). Soil types sampled were similar across all sites, being red Kandosols, with some inliers of less developed Rudosols, all with sandy loam textures [Isbell, 2002; Johnston *et al.*, 2003]. The sites all fall within a broadly similar climatic region characterized by highly seasonal rainfall, with > 90% of the 1,500-1,800 mm of total annual rainfall occurring between November and March.

Our research approach compared intact and transformed (LCLUC) savanna sites, which spanned a range of land uses from native savannas, savannas transformed to C4-dominant stands resulting from severe grassy weed invasion, and savannas transformed into C3 forestry plantations. Three intact savanna sites (hereafter named *savanna1*, *savanna2* and *savanna3*) were all representative of a C3:C4 mixture of a common open-forest savanna assemblage of northern Australia. In all cases the overstory is dominated by *Eucalyptus tetrodonta* and *Eucalyptus miniata*, with a mid-story comprised of semi-deciduous and deciduous small trees and shrubs, and groundcover of C4 grasses [Fox *et al.*, 2001].

To examine savanna transformed towards a C4-dominated endmember by grassy weed invasion, we sampled at two sites (hereafter named *weed1* and *weed2*) where the native savanna vegetation

has been transformed by dramatically altered fire regimes following invasion by the perennial grass *Andropogon gayanus* (Kunth) [Setterfield *et al.*, 2010; Levick *et al.*, 2015]. These transformations have resulted in changes in plant community composition, structure and nutrient cycling [Brooks *et al.*, 2010; Rossiter-Rachor *et al.*, 2009; Rossiter-Rachor *et al.*, 2017]. The two sites were invaded ~14 years prior to sampling, and would have had a similar floristic and structural composition to the three intact savanna sites prior to invasion.

To examine savanna transformed towards a C3-dominated endmember, we sampled two sites located within forestry plantations (hereafter named *forest1* and *forest2*) of *Khaya senegalensis* Desr. (A. Juss., African mahogany), a fast growing, seasonally deciduous/evergreen tree species from the savanna woodlands of north-west Africa [Orwa *et al.*, 2009]. The *forest1* site was planted in 2003 at 560 stems ha⁻¹ [Reilly *et al.*, 2007] and was 13 years old at the time of sampling. *Forest2* was planted in 1972 and was 44 years old at the time of sampling.

2.2 Fire frequency analysis

In order to interpret contemporary patterns of turnover rates of C3- and C4-derived C in the SOC and PyC pools, a detailed survey of site fire history was undertaken using Landsat 5, 7 and 8 scenes for each year from 1990 to the date of soil sampling in 2016. In many years, scenes from more than one satellite were used and were manually assessed primarily in the red/short-wavelength infra-red and near infra-red bands, and when needed red-green-blue (RGB) and thermal bands were used to reduce uncertainty. Identified fire events were classed an ‘early dry season’ if occurrence was up to and excluding 1 June, ‘mid dry season’ up to and excluding 1 July, and ‘late dry season’ on or after 1 July. Frequency was calculated from counts in each class.

2.3 Field sampling and soil core incubation

At each of the seven sites, four to six replicate sampling locations were chosen randomly within a radius of approximately 30 m. Although the sampling locations were randomly positioned, close proximity to tree trunks was avoided in order to avoid large roots. At each location, two soil cores were collected at two different soil depths (0-10 cm, hereafter referred to as ‘shallow’) and 10-30 cm (hereafter referred to as ‘deep’). Surface litter was removed until the mineral soil surface was reached. A small jackhammer was used to drive a custom-made steel corer into the soil with a core barrel allowing pre-cut PVC inserts (inner diameter 100 mm; length 100 or 200 mm) that enabled extraction of intact cores from the steel corer with minimum soil compression. After extraction,

the core's lower end was sealed with a PVC cap and silicone, with small perforations at the base to allow for water drainage.

We collected a total of 63 intact soil cores. Forty-eight were sampled in August 2016 from sites *weed1*, *weed2*, *savanna2*, *savanna3* and *forest2*, while the remaining 15 were sampled in September 2016 from sites *savanna1* and *forest1*. In the laboratory, all cores were initially dried to standardize core water content, and their dry mass and headspace volume were measured. Samples were then incubated over the ensuing 18 months at 32°C in a controlled-temperature room to mimic daytime average temperature of the sampling region. A regular watering regime was instigated that consisted of adding 100 mL of deionized water to each soil core every three days, which roughly corresponded to time of drainage to field capacity and a relatively stable weight.

2.4 Respiration measurements

For each soil core, we measured the respiration rate of CO₂ and carbon isotopic composition of respired CO₂ ($\delta^{13}\text{C}_{\text{CO}_2}$) repeatedly over the course of 18 months. After an initial series of monthly measurements from August 2016 to January 2017, we decreased the measurement frequency to every two months from January to May 2017 and then quarterly from May 2017 to February 2018. During the entire experiment, we obtained between 10 (at *savanna1* and *forest1*) and 11 (at *weed1*, *weed2*, *savanna2*, *savanna3* and *forest2*) CO₂ respiration rate and $\delta^{13}\text{C}_{\text{CO}_2}$ measurement sets for each core. Stable isotope results are reported as per mil (‰) deviations from the VPDB reference standard scale for $\delta^{13}\text{C}$ values.

We conducted the respiration measurements at Charles Darwin University using a Picarro G2101-i cavity ring-down spectrometer, which has an instrumental precision of <0.3‰ (SD) for a 5 min integration time. The Picarro vacuum pump flow rate was $\approx 0.03 \text{ L min}^{-1}$. A continuous, closed air circulation system similar to configuration 3 in *Munksgaard et al.* [2013] was used, where both the inlet and outlet ports of the instrument were connected to a custom-built PVC sampling chamber. The combination of a foam ring and weights placed on top of the soil chamber ensured proper sealing between the chamber and the core to be analyzed. A drying unit (Drierite) was added between the outlet of the soil chamber and the inlet of the Picarro analyzer in order to maintain a low and constant relative humidity level inside the instrument. The system allowed steady build-up of respired CO₂ in the chamber from a start point around atmospheric concentration ($[\text{CO}_2] \approx \sim 400 \text{ ppmV}$) to an endpoint $> 700 \text{ ppmV}$. Each measurement took between 10 minutes and over an hour, depending on the respiration rate of individual cores. Before and after each run, the

instrument was calibrated against two standards: (1) a pressurized cylinder of CO₂ in air (SCOTTY, Air Liquide; 990 ppm CO₂, $\delta^{13}\text{C} = -38.53\text{‰}$) whose $\delta^{13}\text{C}$ value was in turn measured using a GasBench II headspace analyzer coupled to a Delta V^{Plus} (ThermoFinnigan) and (2) an in-house NaHCO₃ powder ($\delta^{13}\text{C} = -6.02\text{‰}$) that was used with a carbonate dissolution module as per the description in *Munksgaard et al.* [2013]. To ensure consistent results across the course of the experiment and because no temporal drift was observed, we chose to apply a single calibration to all our measured $\delta^{13}\text{C}$ values. CO₂ was calibrated using the same pressurized cylinder (SCOTTY, Air Liquide; 990 ppm CO₂).

In the initial stages of the experiment, cores were watered 1-3 days prior to measurement. We later standardized pre-measurement watering so that from January 2017, each core was watered 24 ± 5 hr prior to measurement. In order to compare these undisturbed respirations to disturbed conditions, the last measurement round in February 2018 was conducted after soil samples had been removed from their PVC tube, crushed and homogenized, and then tamped back into their tube.

2.5 Solid phase organic carbon analyses

Solid phase measurements of the soil were made at the end of the incubation experiment.

Representative soil subsamples were taken from each core (15 to 20 g), and samples were ground ($< 100 \mu\text{m}$) and subsequently analyzed at James Cook University for total organic carbon (TOC) and pyrogenic carbon (PyC) content including the $\delta^{13}\text{C}$ value of TOC and PyC ($\delta^{13}\text{C}_{\text{TOC}}$, $\delta^{13}\text{C}_{\text{PyC}}$, respectively).

PyC was analyzed by hydrogen pyrolysis as described in detail in a number of publications [*Wurster et al.*, 2012; *Wurster et al.*, 2013]. Briefly, the solid samples are loaded with a Mo catalyst using an aqueous/methanol solution of ammonium dioxodithiomolybdate $[(\text{NH}_4)_2\text{MoO}_2\text{S}_2]$. In order to give a nominal Mo load of 1% by organic carbon weight, catalyst weight was $\sim 10\%$ of sample weight for all samples. Dried, catalyst-loaded samples were placed in a reactor and pressurized to 15 MPa of hydrogen with a sweep gas flow of 5 L min^{-1} . The reactor was then heated using a pre-programmed temperature profile, initially $300^\circ\text{C min}^{-1}$ to 250°C , then stepped at 8°C min^{-1} until a final hold temperature of 550°C for 2 minutes.

Carbon abundance of TOC and PyC as well as carbon isotope composition ($\delta^{13}\text{C}_{\text{TOC}}$, $\delta^{13}\text{C}_{\text{PyC}}$) were determined using a Costech Elemental Analyzer fitted with a zero-blank auto-sampler coupled via a ConFloIV to a ThermoScientific DeltaV^{PLUS} using Continuous-Flow Isotope Ratio

Mass Spectrometry (CF-IRMS). Because these acidic soils were not expected to have significant inorganic carbon in the form of carbonate minerals, we did not carry out any treatment intended to remove this component. Standard deviations of internal standards were better than $\pm 0.1\%$ for $\delta^{13}\text{C}$ values and $\pm 2\%$ of the value for abundance measurements.

2.6 Data analysis

2.6.1 Two-pool, first-order kinetic decomposition model

We analyzed the kinetics of decomposition during the course of the ~ 1.5 yr incubation, recognizing that the time series of $\delta^{13}\text{C}$ values of respired CO_2 potentially represents an evolving mixture of C derived from different pools. While a simple first-order kinetic decomposition model provided a sufficiently good fit to many of the incubation time series (median $R^2 = 0.89$), a number of cores showed a very poor fit to this model ($R^2 < 0.5$; Appendix S1: Section S1.2, Data S1). These poorly fitting time series also showed a characteristic pattern of decreasing decomposition rate—a pattern reflecting a spectrum of pools with various rates of decomposition [Kuzakov, 2011] that can often be simplified using a two-pool decomposition model [Collins *et al.*, 2000; Fernandez *et al.*, 2003; Wynn *et al.*, 2006; Kuzakov *et al.*, 2014]. In soil samples collected by destructive coring, much of the initial C mineralized may derive from a relatively small pool of highly labile C such as newly fallen litter, root matter, root exudates and even invertebrates or other non-humified biomass C that was disturbed by the coring procedure. We refer to this as the ‘disturbance C’ pool, while the C remaining after this initial pulse can be referred to as the ‘active SOC’ pool. We recognize that the active pool of SOC consists of a mixture of pools with a range of decomposition rates. However, over the time periods of our experiments, the analysis is simplified by assuming a single active pool with an average rate. In order to separate the disturbance C pool from the remainder of the active SOC pool, we used a two-pool, first-order kinetic model to fit to the time series of C remaining during the incubation [Andrn and Paustian, 1987]:

$$F_C(t) = f_{\text{disturbance C}} (e^{-k_{\text{disturbance C}} t}) + (1 - f_{\text{disturbance C}})(e^{-k_{\text{active SOC}} t}) \quad \text{eq.(1)}$$

where $F_C(t)$ is the fraction of carbon remaining at time t , $f_{\text{disturbance C}}$ is the fraction of C in the highly labile disturbance C pool that is mineralized shortly after core disturbance. The remainder ($1 - f_{\text{disturbance C}}$) is the target ‘active’ pool of SOC, and $k_{\text{disturbance C}}$, $k_{\text{active SOC}}$ are the first-order decomposition rate constants for the two pools. Curve fits were calculated using nonlinear least

square curve fitting in MATLAB, producing R^2 of all fits > 0.96 (median $R^2 = 0.994$; Appendix S1: Section S1.2, Data S1). Because it may be argued that these better fits are a consequence of the higher number of parameters in the two-pool models, we calculated Akaike's information criteria (AIC) for both the one-pool and two-pool models (Appendix S1: Section S1.1, Data S1). In order to compare sites using common terms, we recalculated the first-order decomposition rate constants as a half-life. For example, for the active SOC pool, the half-life ($t_{1/2}$) is given by:

$$t_{1/2(\text{active SOC})} = \frac{\ln(2)}{k_{\text{active SOC}}} \quad \text{eq.(2)}$$

2.6.2 Keeling plots and respiration rates

Time series of $\delta^{13}\text{C}$ values and $[\text{CO}_2]$ (approximate sampling frequency of $\sim 1\text{s}^{-1}$) were downloaded from the Picarro log file and further analyzed in MATLAB (MATLAB scripts are provided in the Appendix S1: Section S1.1, Data S2). We generated an automated procedure which first truncates a dataset from a lower endpoint defined by the inflection point of the $[\text{CO}_2]$ time series (usually around 410-430 ppmV) to an upper endpoint of 700 ppmV. These data were then used to plot $\delta^{13}\text{C}$ versus $1/[\text{CO}_2]$, and a linear regression model fitted to derive the intercept ("Keeling plot"; [Pataki *et al.*, 2003]). This procedure was repeated 10,000 times using randomly selected lower and upper endpoints centered on the previously defined values. We used the distribution of these intercept estimates for each core as a measure of uncertainty of the intercept $\delta^{13}\text{C}$ value. The interquartile range was lower than 2‰ for 92% of $\delta^{13}\text{C}$ values measured during the period affected by the disturbance C pool and 96% of those from the active pool. Soil cores with particularly low respiration rates that did not reach 700 ppm after 1 hour of measurement were processed semi-automatically, with the user choosing an upper endpoint corresponding to the last (highest) measured concentration. The time series data were also used to calculate a respiration rate based on the initial linear rise in $[\text{CO}_2]$ calculated from a lower endpoint of 450 ppmV to an upper endpoint of 550 ppmV (see MATLAB script, Data S2). Uncertainties in respiration rates were similarly quantified using a Monte-Carlo approach and 10,000 replications for each measurement. In the Results, we express all uncertainties as the 10th - 90th percentile range for each set of replications.

2.6.3 Solid phase measurements

Solid phase analyses were conducted at the end of the incubation period to minimize soil column disturbance during incubation. These post-incubation measured values are given the designation “final” and are later converted to calculated “initial” values that represent conditions prior to beginning the incubation. $\text{TOC}_{\text{final}}$ represents the sum of $\text{SOC}_{\text{final}}$ and PyC in a sample. Thus $\text{SOC}_{\text{final}}$ was calculated as $\text{TOC}_{\text{final}}$ minus PyC (values reported in %). We used this mass balance to separate the $\delta^{13}\text{C}$ values of the SOC pool ($\delta^{13}\text{C}_{\text{SOC,final}}$), assuming that PyC is stable over the experimental timeframe [Bird *et al.*, 2015], and thus the amount and $\delta^{13}\text{C}$ value of the PyC pool remained constant from beginning to end of the incubation:

$$\delta^{13}\text{C}_{\text{SOC,final}} = \frac{\text{TOC}_{\text{final}} \cdot \delta^{13}\text{C}_{\text{TOC,final}} - \text{PyC} \cdot \delta^{13}\text{C}_{\text{PyC}}}{\text{SOC}_{\text{final}}} \quad \text{eq.(3)}$$

We note that by this definition the SOC pool does not include PyC, but does include a very small amount of the disturbance C pool. Thus, in a subsequent step, we subtract this disturbance C from $\text{SOC}_{\text{final}}$, reducing the C quantity to only the active pool of SOC ($\text{SOC}_{\text{active,final}}$ in % and $\delta^{13}\text{C}_{\text{active SOC}}$ in ‰):

$$\delta^{13}\text{C}_{\text{active SOC,final}} = \frac{\text{SOC}_{\text{final}} \cdot \delta^{13}\text{C}_{\text{SOC,final}} - \text{C}_{\text{disturbance}} \cdot \delta^{13}\text{C}_{\text{disturbance}}}{\text{SOC}_{\text{active,final}}} \quad \text{eq.(4)}$$

where $\text{C}_{\text{disturbance}}$ and $\delta^{13}\text{C}_{\text{disturbance}}$ represent the amount (%) and $\delta^{13}\text{C}$ value of the disturbance pool.

2.6.4 Pre-incubation SOC pool

In order to estimate the amount and $\delta^{13}\text{C}$ values of active SOC prior to incubation, as is used in all of our later calculations and figures ($\text{SOC}_{\text{active,initial}}$ and $\delta^{13}\text{C}_{\text{active SOC,initial}}$), we estimated the amount of active SOC that remained at the end of the incubation using the single-pool, first-order kinetic model applied to active SOC (with a single decay rate constant, $k_{\text{active SOC}}$):

$$F_{\text{active SOC,final}} = e^{-k_{\text{active SOC}}t} \quad \text{eq.(5)}$$

In this equation, F is used as the fraction remaining, and thus begins as a value of 1 at $t = 0$ (*i.e.*, $F_{\text{active SOC,initial}} = 1$) and t is the time of the end of the incubation. We then use this value to estimate the mass of C in the active SOC pool at the beginning of the incubation:

$$m_{\text{active SOC,initial}} = \frac{m_{\text{active SOC,final}}}{F_{\text{active SOC,final}}} \quad \text{eq.(6)}$$

$m_{\text{active SOC,final}}$ is the mass of active SOC at the end of the incubation, and is calculated from the measured value $\text{SOC}_{\text{active,final}}$ and the total mass of dry soil (m_{total}). This is then used to calculate the amount of active SOC at the beginning of the incubation:

$$\text{SOC}_{\text{active,initial}} = m_{\text{active SOC,initial}}/m_{\text{total}} \quad \text{eq.(7)}$$

In a similar fashion, we estimated the initial $\delta^{13}\text{C}$ value of the active SOC pool ($\delta^{13}\text{C}_{\text{active SOC,initial}}$) by accounting for the amount of C and ^{13}C lost through respiration over the course of the incubation using the following equations:

$$\delta^{13}\text{C}_{\text{active SOC, initial}} = \frac{F_{\text{active SOC,final}} \cdot \delta^{13}\text{C}_{\text{active SOC,final}} + C_{\text{resp}} \cdot \delta^{13}\text{C}_{\text{resp}}}{F_{\text{active SOC,initial}}} \quad \text{eq.(8)}$$

where $\delta^{13}\text{C}_{\text{active SOC,final}}$ is the solid phase measurement of the $\delta^{13}\text{C}$ values of active SOC after the incubation obtained using equation 4. The respired amount and $\delta^{13}\text{C}$ value are:

$$C_{\text{resp}} = \int \text{CO}_2 dt \text{ and } \delta^{13}\text{C}_{\text{resp}} = \frac{\sum_{i=1}^n \text{CO}_{2_i} \cdot \delta^{13}\text{CO}_{2_i}}{\sum_{i=1}^n \text{CO}_{2_i}} \quad \text{eqs.(9)}$$

where C_{resp} is the mass of carbon lost through respiration, $\delta^{13}\text{C}_{\text{resp}}$ the total flux-weighted $\delta^{13}\text{C}$ value of respired CO_2 , n the number of data points in each time-series, and CO_{2_i} and $\delta^{13}\text{CO}_{2_i}$ the values of each measurement in the time-series. These integrations were also carried out separately for the period characterized by disturbance C pool as well as the ensuing period lacking the effects of the disturbance C pool, as described below. We use these calculated initial values of the amount and carbon isotopic composition of active SOC in our subsequent discussion, but drop the subscript ‘initial’ (*i.e.*, $\text{SOC}_{\text{active}}$ and $\delta^{13}\text{C}_{\text{active SOC}}$).

2.6.5 Separation of carbon isotopic composition of the active SOC pool

Using constraints from the two-pool kinetic decomposition model described in 2.6.1, the $\delta^{13}\text{C}$ values of CO_2 produced from the active pool of SOC ($\delta^{13}\text{C}_{\text{resp} - \text{active SOC}}$) were isolated from the effects of $\delta^{13}\text{C}$ values of CO_2 produced by decomposition of the disturbance C pool. The $\delta^{13}\text{C}_{\text{resp} - \text{active SOC}}$ values produced during the disturbance period were calculated as the integrated $\delta^{13}\text{CO}_2$ values produced during the period of the incubation period from the initial measurement to the mean lifetime of the disturbance C pool ($1/k_{\text{disturbance C}}$). These portions of the time series of $\delta^{13}\text{CO}_2$ were excluded from subsequent mass balance model calculations of C3- and C4-derived C of the active pool of SOC. This exclusion leaves the remaining $\delta^{13}\text{C}$ time series as a

representation of the active SOC pool ($\delta^{13}\text{C}_{\text{resp} - \text{active SOC}}$), of interest to this study. Thus, $\delta^{13}\text{C}_{\text{resp} - \text{active SOC}}$ was calculated as the remainder, or the integrated $\delta^{13}\text{CO}_2$ time series beyond the mean lifetime of the disturbance C pool. The calculated $\delta^{13}\text{C}_{\text{resp} - \text{active SOC}}$ also excluded the final incubation time period, before which the soils were homogenized.

2.6.6 Separation of SOC, PyC and respired CO_2 derived from C3 and C4 vegetation

We calculated the relative contributions of C3 and C4-derived SOC for the various pools of active SOC, PyC and respired CO_2 . We used a mixing model that considers endmember values of -30.5 and -11.5‰ for C3 and C4 biomass ($\delta^{13}\text{C}_{\text{C3}}$ and $\delta^{13}\text{C}_{\text{C4}}$, respectively; the estimated endmember values are based on the maximum and minimum values observed in the SOC, PyC and CO_2 data sets):

$$f_{\text{C4}} = 1 - f_{\text{C3}} = \frac{\delta^{13}\text{C}_p - \delta^{13}\text{C}_{\text{C3}}}{\delta^{13}\text{C}_{\text{C4}} - \delta^{13}\text{C}_{\text{C3}}} \quad \text{eq.(10)}$$

where f_{C4} and f_{C3} are the fractions of SOC derived from C4 and C3 photosynthesis, respectively, and the subscript 'p' indicates any pool of C partitioned in this way *i.e.*, active SOC, PyC or CO_2 . We note that this mass balance model, in and of itself, does not distinguish between different ages of organic matter, which may be from different sources and may have $\delta^{13}\text{C}$ values from the two endmembers considered.

2.6.7 Fast-C4 and Pyro-C4 indices

To determine whether C4-derived SOC contributes more significantly than C3-derived SOC to the total respiration flux, we calculated a 'fast-C4 index', expressed as the ratio of half-lives for C3 and C4 biomass:

$$\text{Fast C4 index} = \frac{t_{1/2}(\text{C3 active SOC})}{t_{1/2}(\text{C4 active SOC})} \quad \text{eq.(11)}$$

Accordingly, a fast-C4 index > 1 would correspond to an active SOC pool where C4 is being decomposed faster than C3, whereas a fast-C4 index < 1 would correspond to an active SOC pool where C3 is preferentially decomposed.

To assess the relative preservation of C4- and C3-derived C in the PyC pool, we calculated a 'pyro-C4 index', derived from the $\delta^{13}\text{C}$ values of PyC and active SOC such as:

$$\text{Pyro C4 index} = \frac{f_{\text{C4(PyC)}}/f_{\text{C3(PyC)}}}{f_{\text{C4(active SOC)}}/f_{\text{C3(active SOC)}}} \quad \text{eq.(12)}$$

Accordingly, a pyro-C4 index > 1 would correspond to a larger relative contribution of C4-derived C to the PyC pool, whereas a pyro-C4 index < 1 would correspond to a larger relative contribution of C3-derived C to the PyC pool.

3 Results

3.1 Fire history

The fire history of the sites varied significantly, reflecting the history of land use change (Table 1). At the C3-dominant plantation sites (*forest1* and *forest2*), no detectible fire events were observed over the 27-year assessment period. By contrast, across the savanna and weed sites, fires occurred in almost 75% of all years at each site. The weed sites tended to burn later in the dry season with few early dry season fires (frequency of 0.07, 2 in 27 years). The *weed2* site had the highest frequency of late dry season fires (0.52) and *weed1* the highest frequency of mid-season fires (0.38). The *savanna2* site had the highest frequency of early fires and the lowest fraction of late dry season fire, suggesting more intensive seasonal fire management (Table 1). The *savanna3* site had the lowest overall fire frequency, also suggesting intensive fire management by exclusion.

3.2 SOC and PyC content and isotopic composition

The SOC content of all sites was higher in the 0-10 cm cores than in the corresponding 10-30 cm cores (Figure 1A; Friedman's 2-way test, chi-squared = 46.4, $p < 0.001$). The active pool of SOC ranged from 0.9–4% in the 0-10 cm cores and 0.5–1.5% in the 10-30 cm cores, with high intra- and inter-site variability (Figure 1A). The fraction of PyC in the TOC pool (f_{PyC}) was also generally higher in the 0-10 cm cores than in the 10-30 cm cores, but with large inter-site variations (Figure 1B; Friedman's 2-way Test, chi-squared = 7.09, $p = 0.008$). PyC was characteristically low in both the 0-10 cm and 10-30 cm cores of both C4-weed-invaded sites (Figure 1b), with values that typify the 10-30 cm cores elsewhere ($f_{\text{PyC}} < 0.025$).

The $\delta^{13}\text{C}$ values of SOC and PyC showed a predictable pattern of variation with depth, with values that were generally lower (relatively ^{13}C -depleted) in the 0-10 cm cores than in the 10-30 cm cores (Figures 1C,D; Friedman's 2-way test: chi-squared = 33.4 and $p < 0.001$ for $\delta^{13}\text{C}_{\text{SOC}}$ and chi-squared = 12.4 and $p = 0.004$ for $\delta^{13}\text{C}_{\text{PyC}}$). The $\delta^{13}\text{C}$ values of SOC varied between sites of different land use, showing a general trend from higher (relatively ^{13}C -enriched) values for those invaded by C4 Gamba grass (*weed1* and *weed2*; median $\delta^{13}\text{C}_{\text{SOC}}$ -17.9 to -15.0‰) to more ^{13}C -

depleted values for the two C3 forest plantation sites (*forest1* and *forest2*; median $\delta^{13}\text{C}_{\text{SOC}}$ -27.0 to -22.1‰). The mixed C3-C4 savanna sites were intermediate between the weed and forest sites (median $\delta^{13}\text{C}_{\text{SOC}}$ -25.1 to -20.1‰; Figure 1C). The differences between $\delta^{13}\text{C}_{\text{SOC}}$ values of the weed vs. forest sites were significant for both the 0-10 cm and 10-30 cm cores (Friedman's 2-way test: chi-squared = 27.3, $p < 0.001$). A similar trend between sites of different land use was observed for $\delta^{13}\text{C}_{\text{PyC}}$ values (Figure 1D). The differences between the $\delta^{13}\text{C}_{\text{PyC}}$ values of the weed-invaded and forestry plantation sites were also significant for both the 0-10 cm and 10-30 cm cores (Friedman's 2-way test: chi-squared = 33.3, $p < 0.001$). Between the endmembers of the weed and forest sites, the $\delta^{13}\text{C}_{\text{PyC}}$ values of *savanna2* and *savanna3* grouped with the forest sites, while those of *savanna1* were more similar to the weed-invaded sites.

3.3 Carbon isotopic composition of respired CO_2

The trends of $\delta^{13}\text{C}$ values of respired CO_2 ($\delta^{13}\text{C}_{\text{CO}_2}$) over the course of incubation were highly variable and not characterized by consistent trends across sites (Figure 2), except in a small number of cores (see Appendix S1: Section S1.2, Table S2 for trend analysis). For many of the cores from several sites, the pattern might be characterized by high initial variability in $\delta^{13}\text{C}_{\text{CO}_2}$ values during the early portion of the incubation time series, affected by decomposition of the disturbance C pool, followed by more stable or trending conditions thereafter. Cores from three of the sites, showed significant differences in $\delta^{13}\text{C}_{\text{CO}_2}$ values for the final measurement after the sample was homogenized, compared to the rest of the time series representing the active SOC pool (see Appendix S1: Section S1.1, Table S2).

Pooled $\delta^{13}\text{C}_{\text{CO}_2}$ from each site and depth showed a pattern of variation similar to those for the SOC and PyC pools (Figure 1E). Values were generally lower in the 0-10 cm cores than in the 10-30 cm cores, with larger depth-related differences in the savanna and forest sites compared to weed-invaded sites. The lowest $\delta^{13}\text{C}_{\text{CO}_2}$ values were observed in the two C3 forest plantation sites (median $\delta^{13}\text{C}_{\text{CO}_2}$ -29.0 to -20.4‰), highest $\delta^{13}\text{C}_{\text{CO}_2}$ values in the two C4 weed-invaded sites (median $\delta^{13}\text{C}_{\text{CO}_2}$ -14.5 to -12.5‰), and intermediate $\delta^{13}\text{C}_{\text{CO}_2}$ values in the mixed C3-C4 savannas sites (median $\delta^{13}\text{C}_{\text{CO}_2}$ = -22.2 to -18.3‰).

3.4 SOC decomposition

The time series of SOC decomposition for each core showed a generally good fit to a first-order kinetic model with a two-pool structure (R^2 for all fits > 0.96 ; Appendix S1, Data S1). Out of the

64 cores, all but five AIC values were lower for the two-pool models than those for the one-pool models, supporting the choice of basing our analyses on two-pool kinetic models (Appendix S1: Section S1.1, Data S1). The modelled half-life of the disturbance C pool, $t_{1/2(\text{disturbance C})}$, varied from 3–101 days (Figure 3A), but this fraction was generally a small portion of the SOC pool (median $f_{\text{disturbance C}} = 2.3\%$), and always less than 16.5% (Appendix S1: Section S1.1, Data S1). The modelled half-life of the active pool of SOC, $t_{1/2(\text{active SOC})}$, varied from 1.4 to 54.2 yr (Figure 3B). In general, 0-10 cm samples showed a shorter half-life than the 10-30 cm sample (Friedmans' 2-way test: chi-squared = 17, $p < 0.001$), with three sites showing very long half-lives in the 10-30 cm sample (*weed1*, *weed2* and *forest1*). The half-life of active SOC in samples from the two weed-invaded sites was significantly higher than the two nearby savanna sites (*savanna1*, *savanna2*; Friedmans' 2-way test: chi-squared = 21.25, $p < 0.001$).

3.5 Relationship between $\delta^{13}\text{C}_{\text{CO}_2}$, $\delta^{13}\text{C}_{\text{PyC}}$ and $\delta^{13}\text{C}_{\text{SOC}}$

The $\delta^{13}\text{C}$ values of amount-weighted CO_2 produced during incubation of the active SOC pool ($\delta^{13}\text{C}_{\text{C}_{\text{resp}} - \text{active SOC}}$) were closely related to the $\delta^{13}\text{C}$ values of the active SOC pool ($\delta^{13}\text{C}_{\text{active SOC}}$, Figure 4). For all of the 0-10 cm cores from weed-invaded sites, $\delta^{13}\text{C}_{\text{C}_{\text{resp}} - \text{active SOC}}$ values were ^{13}C -enriched relative to $\delta^{13}\text{C}_{\text{active SOC}}$, with a consistent shift away from the 1:1 line of $\delta^{13}\text{C}_{\text{C}_{\text{resp}} - \text{active SOC}}$ vs $\delta^{13}\text{C}_{\text{active SOC}}$ (Figure 4A). In contrast, for the 0-10 cm cores from plantation sites, the $\delta^{13}\text{C}_{\text{C}_{\text{resp}} - \text{active SOC}}$ values were consistently ^{13}C -depleted compared than $\delta^{13}\text{C}_{\text{active SOC}}$, and thus below the 1:1 line. The majority of the 0-10 cm cores from the native savanna sites showed a pattern similar to the weed-invaded sites, although there was more variability across cores and sites. Comparable trends were observed for the 10-30 cm cores, except that five of the cores from forestry sites showed $\delta^{13}\text{C}_{\text{C}_{\text{resp}} - \text{active SOC}}$ values that were higher than $\delta^{13}\text{C}_{\text{active SOC}}$ (Figure 4B).

For most sites, the $\delta^{13}\text{C}$ values of PyC in shallow samples were more ^{13}C -enriched than the $\delta^{13}\text{C}$ values of active SOC, except for the *weed2* site (Figure 5A). Cores from the site with the lowest PyC fraction (*savanna1*) as well as *forest2* had particularly ^{13}C -enriched $\delta^{13}\text{C}_{\text{PyC}}$ relative to $\delta^{13}\text{C}_{\text{active SOC}}$. Trends were different for the 10-30 cm cores, where *savanna3* and the two weed-invaded sites had depleted $\delta^{13}\text{C}_{\text{PyC}}$ values relative to $\delta^{13}\text{C}_{\text{active SOC}}$ values. For other sites, there was no substantial difference between $\delta^{13}\text{C}_{\text{PyC}}$ and $\delta^{13}\text{C}_{\text{active SOC}}$ (Figure 5B).

3.6 Contribution of C4-derived C to respired CO₂ and to the PyC pool

The two weed-invaded sites showed consistently faster decomposition rates for C4-derived active SOC compared to C3-derived, with fast-C4 indices always greater than 1 and as high as 40 (Figure 6). The median half-life of the active SOC pool in weed invaded sites was *ca.* 11.4 yr for shallow cores vs. 22 yr for deep cores, somewhat higher than the values characteristic of the savanna sites (7.0 and 8.4 yr for shallow and deep cores respectively). These differences were significant for the shallow, but not the deep cores (Mann-Whitney U, $p = <0.001$ and 0.82 respectively). The high values for the fast-C4 index at the weed-invaded sites were associated with low values for the pyro-C4 indices (< 1), particularly in the 10-30 cm cores (Figure 7). Opposing trends were observed at forestry plantation sites, with fast-C4 indices often < 1 and pyro-C4 indices often > 1 and up to 2.5 (Figures 6, 7). An exception to this pattern was the deep samples taken from *forest1*, which had fast-C4 indices around 2 and pyro-C4 indices slightly below 1. Similar to weed-invaded sites, the undisturbed native savannas showed somewhat high fast-C4 indices, both in the 0-10 cm and the 10-30 cm samples. At these sites, fast-C4 indices ranged between 1 and 3, except for *savanna1* which had some values < 1 (Figure 6). Variable pyro-C4 indices (*ca.* 0.5-2) were observed for savanna sites, with highest values generally corresponding to high fast-C4 indices, as was the case for *savanna1* (Figures 6, 7).

4 Discussion

4.1 LCLUC effects on the SOC and PyC pools

Given the propensity of Gamba grass invasion to increase fuel loads and fire intensity [Rossiter *et al.*, 2003; Setterfield *et al.*, 2010] and, potentially, net primary productivity, we hypothesized that the two invaded sites would show higher levels of SOC and PyC when compared to the nearby reference savanna sites. Although this was partially supported by data from some weed-invaded samples, others showed smaller pools of active SOC and f_{PyC} compared to savanna samples, and no significant patterns emerged (Figure 1A;B). These observations may reflect the high intensity late-dry season fires that characteristically occur at Gamba grass invaded sites (Table 1; [Setterfield *et al.*, 2010]). First, this may increase susceptibility to runoff and erosion of both SOC and PyC from surface soils [Brooks *et al.*, 2010], and thereby increase lateral transport and removal of PyC by wind and water [Rumpel *et al.*, 2015; Cotrufo *et al.*, 2016; Coppola *et al.*, 2018], exacerbated by the fine nature of grass-derived PyC. In addition, very low PyC values may

result from recombustion of PyC near the surface during intense fires [Saiz *et al.*, 2014]. Of all of the sites studied, *savanna1* showed the lowest f_{PyC} , which may reflect some combination of enhanced erosion or recombustion of PyC.

The size of the active SOC pool at the two forestry sites was relatively similar to each other, and to the native savannas (Figure 1A). The f_{PyC} values likewise did not show a consistent difference between forestry and native savanna sites, but instead a large difference between values at *forest1* compared to *forest2* (Figure 1B). The discrepancy between PyC at the two forestry sites may be attributed to the history of conversion to forestry plantation. Neither site burnt since 1990 (Table 1); the *forest2* site, having been converted to plantation ~30 years prior to *forest1*, may have lost more PyC from the surface soil, presumably via wind and runoff erosion.

In terms of SOC dynamics, Figure 6 suggests that invasion by Gamba grass may, ironically, *increase* the half-life of the active pool of SOC. This effect may simply be due to the increased belowground biomass input from the highly productive invasive grasses, despite their relatively fast decomposition rate. However, as the invasive grass species is known to have more comprehensive effects on the overall ecosystem [Rossiter *et al.*, 2003; Rossiter-Rachor *et al.*, 2009; Ens *et al.*, 2015], the net effects on SOC turnover may also be accomplished through changes in porosity, infiltration, runoff, erosion, microbial and plant diversity, nutrient status, *i.e.*, a multitude of factors derived from the gross modification of the vegetation and soil structure. While the changes to the SOC pool were evident in the C4-grass invaded sites, there were no clear patterns of overall changes to SOC content and turnover in the C3-plantation sites. A more nuanced view of relatively subtle changes in SOC turnover at C3-plantations may be further elucidated by examination of detailed depth distribution and carbon isotopic composition [Guillaume *et al.*, 2015].

Ultimately, we conclude that at the sites studied, LCLUC had no consistent effect on the size of the SOC and PyC pools, but strong effects on SOC decomposition rates by slowing down the decomposition significantly, particularly in deeper soil (10-30 cm sample). In particular, we observed reduced decomposition rates at the C4-weed invaded sites (Figure 3), which may be attributed to factors, which we did not measure in our experiment, such as changes in soil structure and microbial diversity. These observations lend some support to previous work on forest to pasture conversion, which can lead to the preservation of the SOC pool in more stable compounds [Fujisaki *et al.*, 2015; Navarrete *et al.*, 2016]. Such changes in stability may result from changes

in soil structure and microbial diversity that may drive loss of the young, labile portion of SOC and thus reduced mineralization rates [Fujisaki *et al.*, 2015; Hobbey *et al.*, 2017].

4.2 Preferential decomposition of C4-derived biomass under different LCLUC scenarios

The premise of this study is developed on the combined notions that (a) the most labile components of biomass dominate soil microbial respiration (*cf.* [Cotrufo *et al.*, 2013]) and (2) the C4-derived SOC component is significantly more labile than the C3-derived component in native savanna soils [Wynn and Bird, 2007]. Our site selection was designed to extend these two notions to sites with variable LCLUC scenarios involving changes in C3:C4 ratios. Figure 4 shows that for most of the native savanna sites, the respired CO₂ from the active SOC pool was preferentially derived from C4 sources, and thus C4-derived SOC contributed to the total respiration flux more significantly than C3-derived SOC. This observation is generally consistent with the notion of Wynn and Bird [2007] based on the premise that C4-derived SOC may represent decomposition of smaller litter fragments as well as more labile litter compounds than in the C3-derived SOC. The exception to this pattern was *savanna1*, which may be due to the fact that this site had the highest proportion of high intensity late dry season fires across the savanna sites (0.41, Table 1), consuming the active pool of SOC and potentially depriving the soil of C4-derived grass litter input. At the weed-invaded sites, we measured consistently faster rates of decomposition of C4-derived biomass (Figure 4) compared to C3-derived biomass, suggesting that this “fast-C4 effect” is strongly enhanced in sites where the most recent biomass is almost entirely derived from C4 grasses. For the forestry sites planted on native savanna, where almost all of the pre-forest C4-derived SOC has been exhausted, the opposing trend was observed. In this latter LCLUC scenario, and particularly in the shallow cores, the respired CO₂ showed a higher proportion of C3-derived SOC, reflecting the newly added pool of labile organic matter.

We describe this phenomenon in terms of a fast-C4 index (Figure 6), which quantifies the relative rates of decomposition of C4 and C3 biomass. In the C4-weed invaded sites, the higher values of the fast-C4 index observed in deep samples may be attributed to the fact that C3-derived SOC in deeper soil is somewhat older and somewhat more resistant to decomposition [Trumbore, 2000], while shallower depths show a mix of the C4- and C3-derived SOC, still with different decomposition rates but a lesser disparity in the rates for C3 and C4-derived SOC. At forest plantation sites, where the newly introduced vegetation from land use change was C3-derived, the forestry-plantation-derived SOC was faster to decompose, compared to the pre-forestry C4-

derived SOC which had already experienced an extended period of decomposition. Thus, this effect can be related to there remaining only a small amount of old, relatively stable C4-derived SOC in the forestry plantations, particularly in *forest2*. Low values of the fast-C4 index were observed more prominently in the surface samples than in the deep samples, the surface soils being where greater inputs of labile litter have been added from the C3 plantation biomass. A “fast-C4 effect” is not yet observed in the deep soil of the young plantation site (*forest1*), which has only been converted to forestry 13 years prior to sample collection.

In summary, we used the fast-C4 index as a proxy for the lability of the litter produced from recent land-use change. “Fast-C4” sites reflect the introduction of recent C4-derived biomass, while “slow-C4” sites reflect the opposite pattern of recent introduction of C3-derived biomass, where the fast C4 pool has already been partly to largely decomposed. For both LCLUC scenarios, soils preferentially decomposed the newly added pool of labile organic matter, *i.e.* a strong preference for the C4-derived components in the weed-invaded sites and for the C3-derived components in the forestry plantation sites.

4.3 Recent and past vegetation changes in altered savannas

We developed a second index, the pyro-C4 index, to indicate the relative preservation of C4- and C3-derived carbon in the PyC pool. Because PyC is a more stable indicator of older biomass, we hypothesized that the pyro-C4 index may reflect previous ratios of C3:C4 biomass in PyC relative to the current ratio evident from the SOC pool. However, we considered several potential caveats from previous work in native tropical savannas [Saiz *et al.*, 2015], which has shown other effects such as a relative decrease (^{13}C -depletion) of $\delta^{13}\text{C}$ values of PyC compared to overlying vegetation, complicating interpretation of simple C3:C4 ratios. These effects may result from some combination of fractionation during pyrolysis [Leavitt *et al.*, 1982; Das *et al.*, 2010], as well as a potential preservation bias of C3-derived C in the PyC pool. The latter effect may be especially important for coarse-grained PyC particles that remain in situ [Saiz *et al.*, 2015], with finer-grained C4 particles being more susceptible to erosion and transport away from the site of production [Saiz *et al.*, 2018]. We also considered the caveat that for sites where recent (within 10-15 years) Gamba grass invasion has disturbed the fire regime [Brooks *et al.*, 2010], the pyro-C4 index might, in the early stages of invasion, tend to reflect a tendency to initially burn any remaining C3 coarse woody biomass before increasing preference for the preservation of burnt C4

biomass [Commonwealth of Australia, 2015]. In such conditions, we might expect an early stage of low pyro-C4 index, although this was not observed in any of our study sites.

Despite these potential complicating factors, our data suggests that in C4-weed invaded sites, C3-derived biomass dominated the PyC pool, whereas in native savannas and forestry plantations, PyC was predominantly derived from C4 biomass. Undisturbed native savanna sites showed variable pyro-C4 indices that may reflect fire history and management strategies and may show some preference for preservation of C4-derived PyC (values > 1; Figure 7). An illustration of this is *savanna1*, where most samples showed fast-C4 indices near 1 and most pyro-C4 indices > 1. Such pattern may be due to more intensive seasonal fire management strategies employing less intense fires during the early dry season that burn relatively small fuel loads of the C4-dominated understory and produce both relatively low f_{PyC} (lowest values in this data set; Figure 1B), as well as PyC that is dominated by C4-derived carbon. Such practices may also have increased or maintained relatively high woody cover and helped mitigate Gamba grass invasion [Rossiter *et al.*, 2003; Setterfield *et al.*, 2018].

In contrast to native savannas, at C4-weed invaded sites where hot fires result from high fuel loads, C3 biomass was subject to increased mortality both by C4 competition and high severity burning [Setterfield *et al.*, 2010]. Thus this biomass was selectively pyrolyzed, leading to relatively low pyro-C4 indices (values *ca.* 0.5-1; Figure 7). Finally, in forestry sites planted over areas of former savanna vegetation, $\delta^{13}C$ values of PyC reflect the earlier savanna C3:C4 biomass ratio, resulting in relatively high values of the pyro-C4 index (*ca.* 1-2.5). Overall, we conclude that LCLUC had an effect on both the size and nature of the PyC pool, with PyC generally preserving the C3:C4 ratio of the pre-disturbance vegetation.

4.4 A classification scheme for land use change effects on C3:C4 ratios

We develop a framework to systematically assess the effects of recent land use change *versus* prior vegetation composition. Our methodology also provides some recommendations for best practices in future studies on soil core respiration. In particular, we recommend sampling intact cores and excluding the CO₂ produced from the disturbance C pool (see further details in Appendix S1: Section S1.2). The combination of long-term incubation time series with bulk soil data provides an opportunity to develop a classification scheme (Figure 7) to evaluate the effects of LCLUC on the relative rates of decomposition and pyrolysis of C3 and C4 biomass. According to this classification, undisturbed native savanna sites had moderately high values of the fast-C4

index, with variable pyro-C4 indices that reflect fire history and management strategies (e.g., more intensive fire management that burns preferentially the C4-dominated understory). In contrast, weed invaded sites showed a strong preference for selective and rapid decomposition of the newly added C4 component, with higher values of the fast-C4 index, together with the selective pyrolysis of C3 necromass that was the result of invasion, hence low pyro-C4 indices. Lastly, soils from forestry sites had both relatively high values of the pyro-C4 index and relatively low to very low fast-C4 indices, which we attribute to the loss of the native C4 pool over the time since plantation establishment, and to the newly added biomass being dominated by C3-forest litter.

In conclusion, the combination of our fast-C4 and pyro-C4 indices provides a useful framework to distinguish between recent land use change *versus* prior vegetation composition, which could be usefully applied to other tropical settings with mixed C3-C4 vegetation types affected by different LCLUC scenarios.

Acknowledgments

This research was funded by the Australian Research Council Discovery Program (DP160101497), with additional funding to JW from Charles Darwin University EHSE Faculty's Visiting Scholar Program and a National Science Foundation Independent Research and Development Program. The Northern Territory Government and private landowners are acknowledged for access to sampling sites. We also thank Diego Alvarez for fieldwork and laboratory assistance, Matthew Northwood for engineering support, Natalie Rossiter-Rachor for assistance locating and accessing sites and valuable information on Gamba grass, as well as Andrew Edwards and Alex Roberts for analysis of fire histories. Isotopic analyses on soil samples were conducted at James Cook University by Chris Wurster and Rainy Comley. LH, JW, SS and MB conceived the study. CD led data collection and analysis with input from all authors. JW and CD initiated writing of the paper, contributing equally to the writing, with significant input from all authors. The authors declare that they have no conflicts of interest.

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Data Availability

Additional data are available on Zenodo: <https://doi.org/10.5281/zenodo.3757007>

Table 1. Site treatments (natural, converted and transformed savannas), site fire regimes and time since conversion (years). Fire regime was quantified by estimating the fractional fire frequency using a 27-year (1990 to 2016) fire history derived from Landsat imagery. Events were classed as early dry season if occurrence was prior to 1 June, mid dry season from 1 to 30 June, and late dry season on or after 1 July.

Treatment/ Site ID	Location	Fractional Fire Frequency				Time since conversion [yr.]
		Unburnt	Early	Mid	Late	
Natural savanna: mixed C3-C4						
<i>savanna1</i>	13.03597°S, 130.98014°E	0.21	0.07	0.31	0.41	NA
<i>savanna2</i>	13.09862°S, 131.00964°E	0.14	0.31	0.41	0.14	NA
<i>savanna3</i>	12.26562°S, 131.06181°E	0.55	0.07	0.07	0.31	NA
Savanna conversion: plantation forest, C3 dominant						
<i>forest1</i>	12.46941°S, 131.03800°E	1	0	0	0	14
<i>forest2</i>	12.25067°S, 131.04826°E	1	0	0	0	44
Savanna						

transformation: Gamba grass invaded, C4 dominant						
<i>weed1</i>	13.19417°N, 130.93983°E	0.34	0.07	0.38	0.21	10
<i>weed2</i>	13.03740°S, 130.99389°E	0.14	0.07	0.28	0.52	16

FIGURE CAPTIONS

Figure 1. Box and whisker plots of primary solid and gas phase measurements per site and depth.

A) Percent SOC. B) fraction of PyC. C) $\delta^{13}\text{C}$ values of active SOC ($\delta^{13}\text{C}_{\text{active SOC}}$), estimated at beginning of incubation. D) $\delta^{13}\text{C}$ values of PyC ($\delta^{13}\text{C}_{\text{PyC}}$), measured at end of incubation. E) $\delta^{13}\text{C}$ values of CO_2 respired from the active pool of SOC ($\delta^{13}\text{C}_{\text{resp} - \text{active SOC}}$). Boxes represent the 25th -75th percentile range with whiskers extending to the highest and lowest data points exclusive of outliers (denoted by small gray circles and defined as a function of quartile range and whisker length); within each box a circle represents the mean value and a line represents the median value.

Figure 2. Evolution of the individual measurements of the carbon isotopic composition of respired CO_2 ($\delta^{13}\text{C}_{\text{CO}_2}$) for each site and for shallow (left) and deep (right) soil cores. Box and whiskers plots represent the distribution of values based on four to six replicates at each site (min, interquartile range, max) and the circles represent the median value. The grey shaded areas correspond to the range of calculated initial carbon isotopic compositions of the active SOC pool ($\delta^{13}\text{C}_{\text{active SOC}}$) for each site. Vertical bars represent the points in the time series that divide the sequence into an early stage of respired CO_2 that includes the relatively small disturbance C pool with short half-lives (<100 days) vs. the late part of the sequence during which respired CO_2 is dominated by the main pool of active SOC. The labelled numbers indicate the number of individual cores divided in this way at each time point.

Figure 3. Parameters of first-order kinetic decomposition model fit to time series of SOC loss during incubation. Model parameters reported are: half-lives of disturbance C and active SOC pools (A and B, respectively). Each site and depth sample is shown as in Fig. 1. Box and whiskers as defined in Figure 1. Additional parameters and information in Appendix S1: Section S1.2.

Figure 4. Crossplots of $\delta^{13}\text{C}_{\text{active SOC}}$ vs. amount-weighted $\delta^{13}\text{C}_{\text{resp} - \text{active SOC}}$; A) 0-10 cm cores, B) 10-30 cm cores. Square symbols represent amount-weighted CO_2 respired from the main active pool of SOC, while smaller and lighter colored circles represent the amount-weighted CO_2 respired from during the period affected by the disturbance C pool. The 1:1 line is shown for reference. Variability of $\delta^{13}\text{C}_{\text{resp} - \text{active SOC}}$ values between cores, as well as variability over the time-series during incubation is shown in Figure 2.

Figure 5. Crossplots of $\delta^{13}\text{C}_{\text{active SOC}}$ vs. $\delta^{13}\text{C}_{\text{PyC}}$. A) 0-10 cm cores. B) 10-30 cm cores. Analytical errors are within the range of the plot symbols.

Figure 6. Box and whisker plot of Fast-C4 index. Box and whiskers as defined in Figure 1.

Figure 7. Pyro-C4 index vs Fast-C4 index. Squares represent 0-10 cm cores and triangles represent 10-30 cm cores.

Figure 1

Figure 2

Figure 3

Figure 4

Figure 5

Figure 6

Figure 7