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Assessment of vegetation change and landscape variability by using stable carbon isotopes of soil organic matter

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Abstract. Stable carbon isotopic ($\delta^{13}C$) analyses of soil organic matter (SOM) have been used in the past to characterise C3–C4 vegetation changes. However, the temporal and spatial resolution of these isotopic data are not well established. Here, we present data from $\delta^{13}C$ analyses of whole and size-separated SOM, which are discussed in conjunction with organic (total organic carbon (TOC) content) and inorganic (%clay) soil data. These data are put into context with the current vegetation state (assessed from tree size-class distribution) and the 50-year vegetation history (assessed from aerial photographs). By linking below- and above-ground datasets, we show that $\delta^{13}C$ analyses of SOM can accurately record vegetation-change histories over short- (10 and 50 years) and longer-term (hundreds of years) time scales. Our data also show that spatial variability was relatively small for the clay TOC content but was much larger for $\delta^{13}C$ data, indicating that the number of soil cores required for statistical significance is highly dependent on the kind of measurements intended. Finally, interpretation of $\delta^{13}C$ data from SOM to assess the history of C3–C4 vegetation change is complicated by the inherent ^{13}C-enrichment of SOM, owing to decomposition processes, which occurs regardless of vegetation change. We suggest a method for distinguishing ^{13}C-enrichment of SOM that is due to soil-inherent (decomposition-related) processes from ^{13}C-enrichment that is due to increased inputs of C4 organic matter.

Introduction

Savannas are dynamic ecosystems and highly sensitive to environmental pressures such as grazing, fire and climate. Accordingly, the composition of savanna vegetation can fluctuate between a more woody state and a more grassy state. Changes in vegetation composition can occur within several years, for example, owing to a serious drought, rapidly increased grazing pressure or a change in fire frequency (e.g. Archer 1989; Fensham and Holman 1999; Archer *et al*. 2001; Ward *et al*. 2001; Burrows *et al*. 2002; Fensham *et al*. 2003; Sharp and Whittaker 2003). In the longer term, oscillation of such short-term vegetation changes results in a vegetation system that varies around a more or less steady average. Several studies suggest that recent, human-induced changes can shift an ecosystem towards a new vegetation state. On a local scale, the change from Aboriginal land management to practices introduced to Australia from Europe, where domestic livestock grazing and road infrastructure led to fire suppression (Ward *et al*. 2001), has been linked to the spread of woody vegetation over the last 100 years. On a global scale, increased carbon dioxide concentration in the atmosphere since industrialisation has led to 'CO_2 fertilisation', causing shifts in drought tolerance and plant growth rates (Bond and Midgley 2000). Specifically, a human-induced change in vegetation composition tends to shift 'remnant' savanna ecosystems from a more open to a more woody state. This type of vegetation change has been reported in other parts of the world and has been termed 'thickening', 'woody weed invasion' or 'woody encroachment' (Boutton *et al*. 1998; Archer *et al*. 2001). The common method of measuring an increase in woody vegetation is either by recording the change in tree basal area by field surveys (Burrows *et al*. 2002) or by measuring the tree cover from aerial photography (Fensham *et al*. 2003). An increase in tree basal area has been interpreted by some as evidence for vegetation thickening (i.e. a shift from a long-term average savanna state to a woodland system, coinciding with the introduction of European land-management practices and rise in atmospheric CO_2 concentrations; Burrows *et al*. 2002). Others, however, believe that the increase in woody plant basal area is simply a segment of the natural growth–regrowth cycles (governed

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by droughts and fire) (Fensham and Holman 1999; Fensham *et al*. 2003). $\delta^{13}C$ and ^{14}C analyses of soil organic matter (SOM) have been used to determine whether a change from a grassland-dominated to a woodland-dominated ecosystem has taken place and to estimate the relative timing of that vegetation change (Boutton *et al*. 1998; Krull *et al*. 2005). While vegetation-change records derived from aerial photographs or measurement of changes in tree basal area are limited to the last 50 years or less, $\delta^{13}C$ analysis of whole and size-separated SOM can achieve a greater resolution of vegetation-change processes, allowing the documentation of vegetation changes over short- (50–100 years) and longer-term (hundreds of years) time scales.

The application of $\delta^{13}C$ analyses to vegetation-change studies is currently limited to ecosystems where most of the grasses are represented by species with the C4 photosynthetic pathway. C4 plants (most tropical grasses) have an average $\delta^{13}C$ value of $-13‰$ and C3 plants (trees, woody shrubs, temperate grasses) an average of $-26‰$ (Deines 1980). This isotopic difference between tropical grasses (C4) and trees (C3) makes it possible to distinguish grass from woody plant-derived organic matter. The change in proportion of woody and grassy material over time can be estimated from $\delta^{13}C$ analyses of whole and size-separated SOM down the soil profile (Boutton *et al*. 1998; Krull *et al*. 2005). The relative proportions of derived soil organic carbon from grasses and woody plants are commonly estimated from the following equation developed by Ludlow *et al*. (1976):

$$\%C4 = ((\delta_s - \delta_3)/(\delta_4 - \delta_3)) \times 100, \qquad (1)$$

where δ_s, δ_3 and δ_4 are the $\delta^{13}C$ values of the sample and the $\delta^{13}C$ values of the average C3 (woody plant) and C4 (grass) plants. This equation can be applied with confidence to litter and surface SOM $\delta^{13}C$ values; however, estimates from soil samples from deeper horizons are associated with greater uncertainty because of isotopic fractionation processes during decomposition (Balesdent and Mariotti 1996; Krull and Skjemstad 2003). Specifically, the difficulty lies in the often-observed ^{13}C-enrichment of SOM with depth, owing to fractionation processes unrelated to C3–C4 vegetation change. The range of this soil-inherent ^{13}C-enrichment can vary between 1‰ in arid and weakly developed soils and up to 5‰ in wet tropical, well developed soils (Garten *et al*. 2000; Krull *et al*. 2002). Although it is not precisely known what cause or combination of causes underpin the ^{13}C-enrichment, most studies regard microbial isotopic fractionation as well as the contribution of ^{13}C-enriched root material as the most influential factors. More comprehensive reviews about this topic can be found in Boutton (1996), Balesdent and Mariotti (1996), Ehleringer *et al*. (2000), Šantrůćková *et al*. (2000), Bird *et al*. (2003) and Krull and Skjemstad (2003). Consequently, in ecosystems that constitute a mix of C3 and C4 plants (opposed to monocultures), such as savannas and open woodlands, care is required to correctly assess the history and identify the long-term trends of vegetation change. Furthermore, the dynamic nature of these ecosystems (e.g. fire, drought) may result in large above- and below-ground variability of organic and inorganic components.

We chose a study area that had undergone recent vegetation change from a more open to a more thickened state in order to

- assess the spatial variability of soil organic and inorganic properties at two sites (a woody and a more open site),
- evaluate how $\delta^{13}C$ values of SOM from bulk and size-separated soil fractions can be used to determine the relative timing and degree of vegetation change and
- illustrate how aerial photography, tree size-class distribution and isotopic analyses can be used to distinguish short-term vegetation dynamics (e.g. resulting from recent drought) from longer-term changes.

Materials and methods

Location and site history

The study area ('Blue Range') is located 130 km north-west of Charters Towers, Queensland, Australia (19°09′S, 145°24′E), and is situated within the catchment of the Burdekin River. The climate is semi-arid tropical, with hot, wet summers and warm, dry winters. Mean annual rainfall is 660 mm, mainly during December–March, and the annual evaporation is 2035 mm. The mean annual temperature is 23.8°C, with daily maximum and minimum temperatures of 35°C and 21°C in December and 25°C and 11°C in July. The main vegetation type is eucalypt woodland.

The study site is an uncleared, remnant woodland, consisting of a mosaic of small, relatively open grassy areas ('Open site') and more densely wooded areas ('Tree site') (Fig. 1). The site is used as a horse paddock and to graze stud cattle. The Tree site is located on a slight ridge and the Open site on a gentle slope. On the basis of information provided by the landholder, the tree density of the paddock had significantly increased over the last 40 years from large, widely spaced trees to dense areas of trees that now limit visibility. The landholder became conscious of the proliferation of trees ('Thickening') in the 1970s and 1980s. Severe droughts during the mid-1980s and early 1990s killed several trees and left the soil surface bare for some years. Since then, the tree density has continued to increase. Burning of the site occurs generally every 3–4 years, with the last fire in 1999. The site was sampled in June 2002.

Soil collection and vegetation assessment

At the Tree site, 25 cores were taken within a 50×50 m site (12.5 m between cores). The Open site was located approximately 100 m north-east of the Tree site. A total of five cores were taken at the Open site along a 64-m-long transect, with 16 m between each core. The rationale for taking a greater number of samples at the Tree site than at the Open site was two-fold. First, Veldkamp and Weitz (1994) estimated that the $\delta^{13}C$ variance in mixed C3–C4 systems was an order of magnitude greater because of the larger heterogeneity than in systems with one dominant vegetation type. Therefore, more samples are necessary in mixed tree–grass systems to ensure statistical significance. Second, it proved to be difficult to locate an Open site of similar extent as the Tree site, with the same basic vegetation composition and similar soil type as the Tree site.

Soil cores were divided into seven depth intervals (0–5, 5–10, 10–20, 20–30, 30–50, 50–70 and 70–100 cm). In the case of relatively

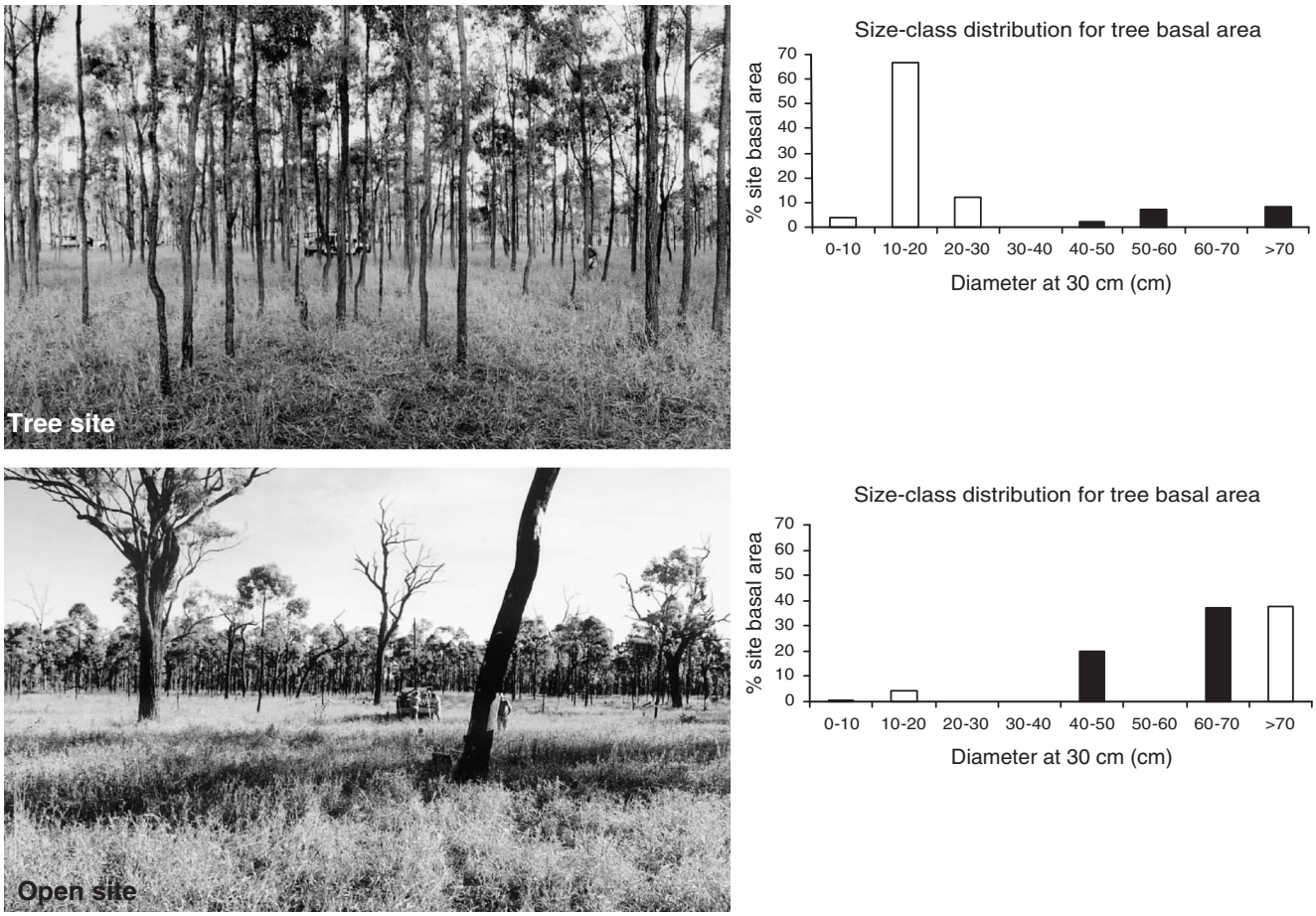

Fig. 1. Photographs and tree size class distributions are shown as a percentage of site basal area for the Tree and the Open sites. White and black bars are live and dead trees, respectively.

recent (<100 years) vegetation changes, it was expected that the most pronounced isotopic changes would occur in the uppermost 5 cm. Consequently, to ensure adequate resolution of this interval, a separate sample of the uppermost 0–2 cm was collected immediately adjacent to the main core. Litter samples were collected adjacent to the core. Fresh foliage and roots of herbaceous species of each of the grass, forb and tree species were also sampled, washed, dried and homogenised for isotopic analysis.

Stand tree basal area was measured at 130 cm by the Bitterlich technique (Fig. 2; Bitterlich 1948 cited in Grosenbaugh 1952). Each counted tree was identified to species and determined whether it was alive or dead. Measurements were taken at 15 core positions in the Tree site and at the five core positions in the Open site. The tree size-class distribution as a proportion of total site basal area was derived from the stem circumference at 30 cm for the two trees closest to each core position. The tree size-class distribution was corrected for the proportion of live and dead basal area by using the tree stand basal area corrected to 30 cm:

$$BA_{30\,cm} = 1.5294 \times BA_{130\,cm}, \quad (2)$$

where $BA_{30\,cm}$ is the stand basal area ($m^2\ ha^{-1}$) at 30 cm by the TRAPS methodology and $BA_{130\,cm}$ is the stand basal area ($m^2\ ha^{-1}$) at 130 cm by the Bitterlich technique. The relationship between stand basal area at 130 and 30 cm was developed from 20 woodland sites. Live-stand basal area was estimated by both the TRAPS woodland monitoring methodology (Burrows *et al*. 2002) that measures individual trees at 30 cm within a specific area and the Bitterlich method at 130 cm. Equation 2 assumes that the relationship can be applied to various live and dead species within a stand.

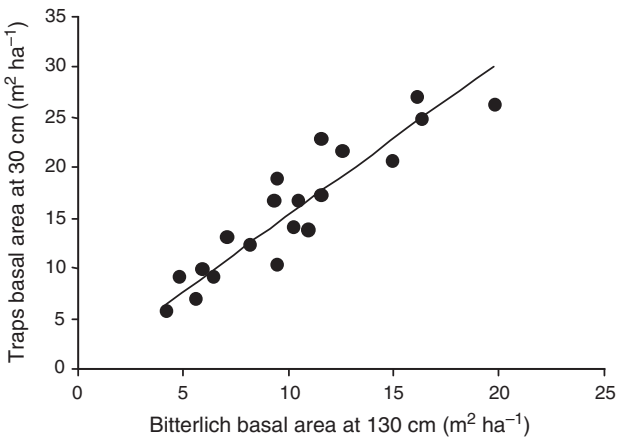

Fig. 2. Relationship between live tree stand basal area measured at 30 cm by using the TRAPS technique ($BA_{30\,cm}$) and live tree stand basal area at 130 cm by using the Bitterlich technique ($BA_{130\,cm}$). $BA_{30\,cm} = 1.5294 \times BA_{130\,cm}$; $R^2 = 0.84$, $P < 0.001$.

Tree biomass was estimated by applying biomass allometrics (Burrows *et al*. 2000) to each individual tree measured in the size-class distribution dataset. A correction factor was applied for back transformation according to Baskerville (1972). Biomass of the standing dead trees was derived for each tree by discounting the calculated live tree biomass (divided by a correction factor of 2.07; Burrows *et al*. 2002) for loss of canopy structure. This approach assumes that only the biomass of the trunk is left in dead trees, which is probably an underestimate as often some large branches remain, particularly in trees that have died recently. The biomass of these trees was scaled up to the stand basal area on the basis of the proportion of live and dead tree basal area. Grass and litter biomass was determined from cut and dried (at 60°C) samples. Overstorey cover was assessed along transects at 100 points in the Tree and at 60 points in the Open site by using the vertical tube method (Johansson 1985) and a gimballed sighting-tube instrument (Hassett *et al*. 2000). Understorey cover was assessed at these same points.

Sample processing and preparation for $\delta^{13}C$ analyses

Litter and vegetation samples were dried at 60°C, cleaned of any adhering soil material, and finely ground. Soil samples were dried at 45°C and ground to pass a 2-mm screen. The >2-mm soil fraction was retained and sticks, coarse (>1 mm diameter) and fine roots (<1 mm diameter) were separated by hand from the pebble fraction, finely ground and saved for $\delta^{13}C$ analysis. The <2-mm fraction was split into subsamples. One set of subsamples was used for $\delta^{13}C$ analyses, elemental carbon and mid-infrared analyses of each core. For another set of subsamples, each horizon was bulked for organic matter size separation and $\delta^{13}C$ analyses.

Elemental analyses for total carbon

Soil samples were analysed for total organic carbon TOC content by using a LECO C 144 carbon analyser as described by Merry and Spouncer (1988). Prior to analysis, each soil sample was tested with HCl for the presence of carbonate. The test was negative for all samples.

Size separation of the <2-mm fraction

The procedure used for separation of particulate organic carbon (POC) was similar to that described by Cambardella and Elliott (1992). Soil samples (10 g) were shaken overnight with 40 mL of 0.5% sodium hexametaphosphate solution. The suspended material was passed through a 200-µm sieve over a 53-µm sieve. The sediment, consisting of sand and particulate organic matter, was gently worked with a spatula to ensure that no aggregates were retained in the particulate fractions.

$\delta^{13}C$ analysis

For stable isotopic analyses, a sample mass yielding between 300–800 µg carbon was placed into ultraclean tin capsules and sealed. Samples were combusted and analysed for $\delta^{13}C$, $\delta^{15}N$ (not reported here) and total N (TN) on a 20–20 Europa Scientific automated nitrogen carbon analysis mass spectrometer (ANCA-MS). Every fourth sample was analysed as a replicate. Isotope results are reported in the conventional δ notation as ‰ relative to the carbon isotopic ratio of the V-PDB standard (Peterson and Fry 1987). EDTA (Australian National University: 41.10% C, 9.59% N, $\delta^{13}C$ −32.0‰, $\delta^{15}N$ −5.5‰) and an internal reference mix (ammonium sulfate: 34.00% C, 4.25% N, $\delta^{13}C$ −10.5‰, $\delta^{15}N$ −1.4‰) were used as internal standards. Standard deviation from dual replicates was 0.2‰ at the Open site and 0.4‰ at the Tree site.

Fourier transform infrared (FTIR) spectroscopy and partial least squares (PLS) analysis

Janik *et al*. (1995, 1998) demonstrated that by application of PLS analysis, FTIR spectra can be used to model soil properties. Infrared spectra were collected on a rapid-scan Bio-Rad FTS-175 FTIR spectrometer equipped with a Peltier-cooled DTGS detector and extended range KBr beam-splitter, scanning at 8 cm^{-1} resolution and a 0.5 s scan rate to give a spectral range of 8000–470 cm^{-1}. The instrument compartment and sample chamber were purged with H_2O- and CO_2-free air to remove atmospheric effects. Powdered samples were poured into 10-mm diameter aluminum cups and the top surfaces levelled. Spectra were recorded for infrared measurements from 4000 to 500 cm^{-1} at 1.92-cm^{-1} intervals and a resolution of 4 cm^{-1}. The spectral operating software, WIN_IR Version 4.0, a variant of the Galactic (NH) GRAMS-32 operating system, was provided as the standard operating system for the spectrometer. The partial least squares (PLS) method was based on algorithms published by Haaland and Thomas (1988) and incorporated into PLSplus/IQ Version 3.0.

Aerial photography

Aerial photos for five dates (1951—oldest aerial photo available, 1964, 1980, 1991 and 2002) were rectified against one another by using GIS (Erdas Imagine Version 8.7) to ensure the same site areas were identified in each photo. Tree canopy cover was estimated by assessing an average of 270 and 420 points (in a grid pattern) for the Open and Tree sites respectively at each date. Visual interpretation was required for canopy classification from the aerial photos because of differences in contrast, blurriness and shadowing between photo dates. Data were standardised to 100% for the state depicted in the 1951 photograph.

Statistical analyses

A *t*-test was used to assess treatment effects at each depth with GenStat (6th edition). Variances were tested for equality.

Results

Vegetation composition and $\delta^{13}C$ data

The vegetation composition of the Tree and Open sites in June 2002 is illustrated in Fig. 1 and summarised in Tables 1 and 2. Vegetation at both sites consisted of a mix of C3 and C4 species (Table 1) and was dominated at the Tree site by thin narrow-leaf ironbark (*Eucalyptus crebra*) trees (C3) with a few larger, older trees interspersed, many of which were dead (mortality presumably due to severe droughts in the mid-1980s and early 1990s). Groundcover was dominated by *Urochloa* (*Urochloa mosambicencis*) and *Chrysopogon* (*Chrysopogon fallax*) grasses (C4). The Open site contained widely spaced (20–30 m) large narrow-leaf ironbark trees, many of which were dead or ailing, and a few smaller trees. Groundcover was dominated by *Urochloa* and *Chrysopogon*. A small proportion (<2% in the Tree site and <1% in the Open site) of the ground layer was composed of C3 forbs (*Sida* spp., *Stylosanthes scabra*, *Grewia latifolia*, *Crotalaria verrucosa*) with $\delta^{13}C$ values of the leaves averaging −29.0‰ (Table 1). Leaves and twigs of the forbs and woody C3 vegetation were ^{13}C-depleted (average −28.0‰) compared with the bark (average −27.1‰), and there was a ^{13}C-enrichment in roots (−2.0‰ and −2.3‰ for trees and forbs, respectively) compared with leaves, which

Table 1. Species composition, photosynthetic pathway (C3 or C4), mean relative cover (%) at each site and $\delta^{13}C$ values (‰) of plant elements
T = Tree site and O = Open site

Species	Common name	Photosynthetic pathway	Occurrence (T, O); % of groundcover (grasses only)	$\delta^{13}C$ (‰) of plant material
Eucalyptus crebra	Narrow-leaf ironbark (tree)	C3	T, O	−27.1 (bark) −28.4 (leaves, twigs – mature trees) −27.9 (leaves, twigs – regrowth) −26.0 (roots)
Eucalyptus brownii	Reid River Box (tree)	C3	T	−28.1 (leaves, twigs)
Eucalyptus clarksoniana	Bloodwood (tree)	C3	T	−27.4 (leaves, twigs)
Urochloa mosambicensis	Urochloa	C4	T (45%), O (49%)	−13.5 (leaves) −12.9 (roots)
Chrysopogon fallax	Golden beard grass	C4	T (45%), O (49%)	−12.8 (leaves) −13.0 (roots)
Heteropogon contortus	Black speargrass	C4	T (5%), O (1%)	−13.3 (leaves) −13.1 (roots)
Aristida spp.	Wiregrass	C4	T (3%)	−13.9 (leaves) −14.3 (roots)
Sida spp.	Sida	C3 forb	T (2%), O (<1%)	−29.8 (leaves) −27.5 (roots)
Stylosanthes scabra	Stylo	C3 forb	T (<1%)	−28.8 (leaves)
Crotalaria verrucosa	Rattlepod	C3 forb	T (<1%)	−28.0 (leaves)

Table 2. Tree basal area at 30 cm ($m^2\ ha^{-1}$), estimated biomass ($t\ ha^{-1}$), overstorey and understorey cover (%) for the Tree and Open sites

Parameter	Tree site	Open site
Alive basal area at 30 cm ($m^2\ ha^{-1}$)	11.8	2.4
Dead basal area at 30 cm ($m^2\ ha^{-1}$)	2.8	3.7
Tree biomass alive ($t\ ha^{-2}$)	49.3	21.1
Tree biomass dead ($t\ ha^{-2}$)	8.5	12.7
Grass biomass ($t\ ha^{-2}$)	1.4	1.5
Litter biomass ($t\ ha^{-2}$)	3.2	2.5
Total aboveground biomass ($t\ ha^{-2}$)	62.4	37.8
Overstorey cover		
Green leaf (%)	35	2
Branch (%)	4	2
Sky (%)	61	96
Understorey cover		
Green leaf (%)	35	65
Litter (%)	60	30
Course woody debris (%)	1	–
Bare soil (%)	4	5

is a common observation in plants (e.g. Leavitt and Long 1986; Wedin *et al*. 1995; Bird *et al*. 2003) (Table 1). All grasses were identified as having the C4 photosynthetic pathway, with *Aristida* (−13.9‰) having the most ^{13}C-depleted and *Chrysopogon* having the most ^{13}C-enriched values (−12.8‰). The isotopic differences between roots and shoots were <0.6‰ (Table 1). Litter yields at the Tree site and Open site were 3200 and 2500 $kg\ ha^{-1}$, respectively. Proportions of grass- and tree-derived components (based on weights of separated materials) were about equal at the Tree site and were dominated by grass litter at the Open site. Total aboveground biomass was 1.7 times and tree basal area was almost five times greater at the Tree site than at the Open site (Table 2).

Soil properties and $\delta^{13}C$ data

Soil types at the Open and Tree sites differed only slightly, with the soil type at the Open site being classified as a mottled-sodic, eutrophic Yellow Chromosol and the soil type at the Tree site as a mottled-sodic, eutrophic Brown Chromosol (Isbell 2002) (Table 3). Both sites were situated on a slightly undulating Quaternary alluvial plain with 0.5–1% slope. Permeability and drainage were moderate and termite mounds are common. Clay content increased with depth and the soil structure changed from massive to angular-blocky. Soil pH was 7.0 at the Open site and slightly more acidic (average pH 6.5) at the Tree site (Table 3). FTIR-predicted values for %clay and TOC content are illustrated in Fig. 3. There were no significant differences between the Tree site and the Open site in clay and TOC contents (Fig. 3*a*, *b*). Standard deviations for clay content tended to be similar within the soil profile and were largest in the uppermost soil horizons for TOC contents.

The distributions of $\delta^{13}C$ values of whole SOM and associated standard deviations at each site are illustrated in Fig. 4. Standard deviations of $\delta^{13}C$ values were of similar extent to the TOC contents, attesting to the spatial

Table 3. Soil profile descriptions from a representative core each from the Tree site and the Open site

Soil profile description of Tree site: eutrophic Brown Chromosol		
Soil surface: Hard setting, no coarse fragments; no evidence of erosion		
Horizon:	Depth (cm)	
A1	0.0–10.0	Brown (10YR53) moist; loamy fine sand; platy weak 2–5-mm structure; no segregations; hard dry; no pan; pH 6.0
A12	10.0–30.0	Brown (7.5YR54) moist; clayey fine sand; massive structure; no segregations; hard dry; no pan; pH 6.5
B1	30.0–60.0	Light brown to reddish yellow (7.5YR65) moist; fine sandy loam; massive structure; no segregations; hard dry; no pan; pH 6.5
B21	60.0–90.0	Strong brown (7.5YR56) moist; common 10–20% medium 5–15-mm distinct reddish brown mottles; sandy clay loam; few 2–10% medium gravelly 6–20-mm rounded quartz; massive; few 2–10% coarse 6–20-mm ferromanganiferous nodules; hard dry; no pan; slightly soapy feel; pH 7.0
B22t	90.0–120.0	Brown to dark brown (7.5YR42) moist; many 20–50% medium 5–15-mm faint brown and fine <5-mm distinct black mottles; light medium clay; angular blocky moderate 10–20-mm structure; firm moist; no pan; soapy feel
Soil profile description of Open site: eutrophic Yellow Chromosol		
Soil surface: Firm, no coarse fragments; no evidence of erosion		
Horizon:	Depth (cm)	
A1	0.0–10.0	Pale brown (10YR6/3) moist; loamy fine sand; massive structure; no segregations; hard dry; no pan; pH 7.0
A12	10.0–30.0	Brown (10YR5/3) moist; clayey fine sand; massive structure; no segregations; hard dry; no pan; pH 7.0
B1	30.0–70.0	Light brown to reddish yellow (7.5YR6/5) moist; few 2–10% fine <5-mm prominent black mottles; fine sandy loam; massive structure; no segregations; hard dry; no pan; pH 7.0
B21	70.0–90.5	Reddish yellowish (7.5YR6/6) moist; common 10–20% fine <5-mm distinct black mottles; sandy clay loam; angular blocky 5–10-mm structure; very few <2% medium 2–6-mm ferromanganiferous concretions; firm moist; no pan; pH 7.0
B22t	90.5–120.5	Dark greyish brown (10YR4/2) moist; many 20–50% medium 1–15-mm faint brown and fine <5-mm distinct black mottles; light medium clay; angular blocky moderate 10–20-mm structure; firm moist; no pan

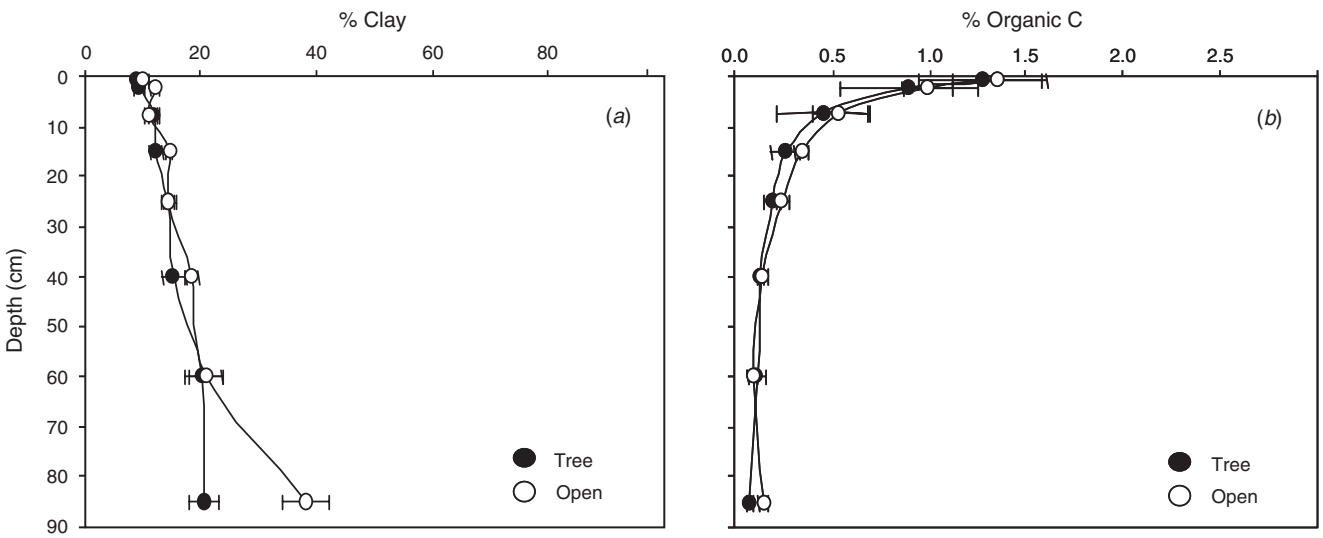

Fig. 3. Depth distribution of (*a*) FTIR-predicted distributions of clay content (%) and (*b*) LECO-determined TOC content at the Tree and Open sites. Error bars indicate the variation within sites.

variability of $\delta^{13}C$ values of SOM in mixed tree–grass systems (Veldkamp and Weitz 1994; Bowman and Cook 2002; Bird *et al*. 2002; Krull *et al*. 2005). At the Tree site, $\delta^{13}C$ values steadily increased in the uppermost 30 cm from an initial value of −22.5‰ in the litter and soil surface to −17.5‰ below that depth. The Open site showed a similar, yet more ^{13}C-enriched trend, than the Tree site. A notable exception was the large difference in the $\delta^{13}C$ values between the litter (−15.1‰) and soil surface (−18.7‰) (Fig. 4).

The $\delta^{13}C$ results of the POC fraction (POC: >200 + 53–200 µm) in comparison with the whole soil are shown in Fig. 5 (litter layer excluded). The relative proportions of the POC fractions in each depth increment are summarised in Table 4. SOM was dominated by the <53-µm fraction, except for the uppermost 5 cm, and the Open site had a greater

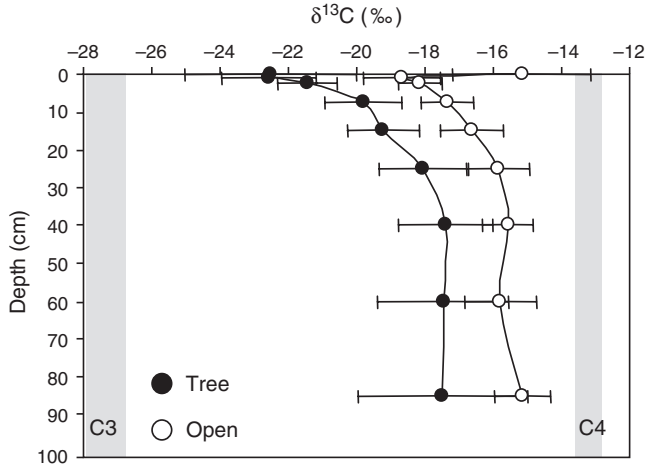

Fig. 4. $\delta^{13}C$ values of whole SOM from the Tree and Open sites. The error bars indicate the variation within each site and the differences between the two sites are significant ($P \leq 0.001$) to a depth of 30 cm and not statistically different below that depth ($P > 0.001$). The shaded zones delineate the breadth as well as average $\delta^{13}C$ value for each C3 and C4 plant species (isotopic endmembers) (Table 1).

proportion of the POC fractions than the Tree site. At the Tree site, $\delta^{13}C$ values of the POC fraction were similar to the whole SOM values only in the uppermost 5 cm and deviated below that depth. The POC fraction had $\delta^{13}C$ values that were largely representative of a C3-type vegetation; however, the >200-µm fraction was much more variable than the 53–200-µm fraction. By comparison, $\delta^{13}C$ values of the POC pools at the Open site were more similar to whole SOM values, both having greater ^{13}C-enrichment than the Tree site.

Aerial photography

Analyses of the aerial photos indicated that in 1951 and 1964, large trees dominated both the Tree and Open sites; however, in 1980, large tree canopies declined and small tree canopies were increasing at the Tree site but not at the Open site. This trend (decline of large tree canopies and increase in small tree canopies) continued in the following years at the Tree site.

Discussion

Clay content did not differ significantly between the Tree and Open sites, suggesting that the observed changes in vegetation and SOM $\delta^{13}C$ values were not driven by differences in soil structure. On the basis of the standard deviations, the number of samples (cores) required for statistically significant determination of inorganic soil properties (clay content) is less than the number required for the organic properties. The greater standard deviation in TOC than clay content attests to the larger variability in organic than in inorganic soil components (Fig. 3*a*, *b*). Furthermore, the greater standard deviation in upper than in lower horizons reflects the spatially variable litter and root input from trees and grasses.

The $\delta^{13}C$ data of SOM suggest that the current vegetation state of the Open site (i.e. the C4-dominated litter material) is not likely to be representative of its 50–100-year history, as the $\delta^{13}C$ values between litter and surface soil (0–2 and 0–5 cm) are significantly different from one another (Fig. 4). In a steady-state soil-vegetation system, $\delta^{13}C$ values between litter and soil surface tend to be very similar (Balesdent *et al*. 1993; Boutton 1996; Boutton *et al*. 1998; Balesdent and Mariotti 1996), and Huang *et al*. (1997) and Schweizer *et al*. (1999) did not find a significant change in $\delta^{13}C$

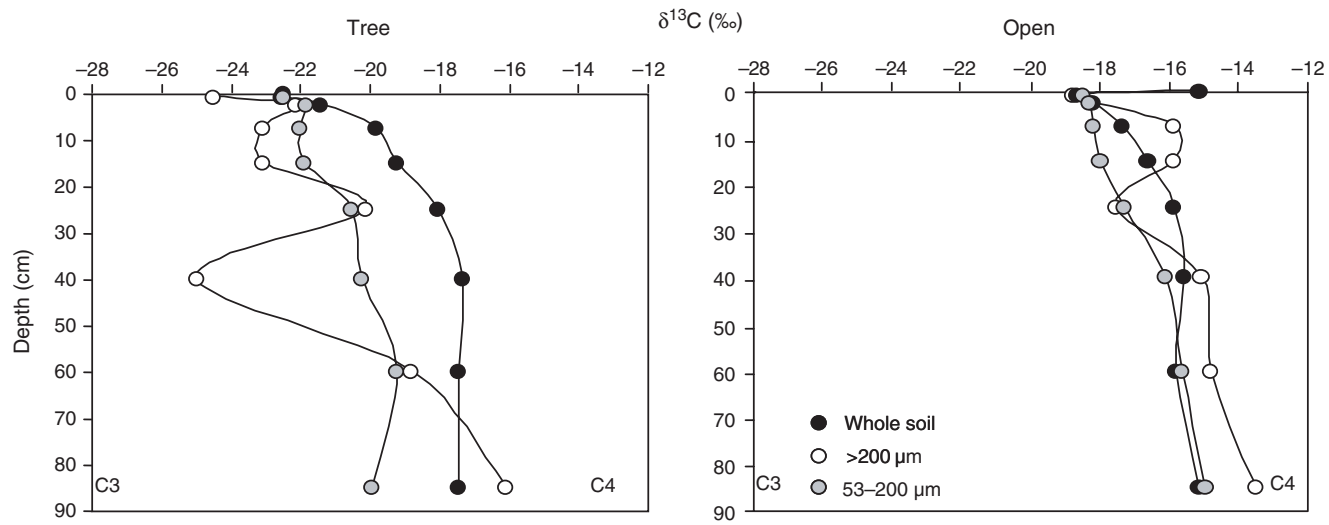

Fig. 5. $\delta^{13}C$ values of POC fractions (>200 and 53–200 µm) and whole SOM from the Tree and Open sites.

Table 4. Relative proportions (%) of SOM in the >200, 200–53 and <53-μm ('humified') fractions at the Tree and Open sites

The data show that, except for the uppermost 5 cm, most of the SOM is contained in the more humified, <53-μm fraction. No standard deviations are given as size separations were done on bulked samples

Depth (cm)	Tree site			Open site		
	% >200 μm	% 53–200 μm	% <53 μm	% >200 μm	% 53–200 μm	% <53 μm
0–2	23.5	21.1	55.4	29.6	46.8	23.6
0–5	20.8	28.0	51.2	45.3	40.7	14.0
5–10	8.1	10.0	81.9	10.0	14.1	75.9
10–20	3.9	8.1	88.0	8.1	9.1	82.7
20–30	2.9	4.6	92.5	6.8	5.2	88.0
30–50	4.6	3.5	91.9	6.2	3.2	90.6
50–70	4.0	3.0	93.1	4.3	2.1	93.7
70–100	1.1	3.0	95.6	5.7	2.5	91.9

values in litter incubation (after 119 days) and field studies (after 23 years).

The relative timing of vegetation changes can be assessed by comparing the $\delta^{13}C$ values of the POC pool with those from the bulk SOM (represented largely by the <53-μm fraction; Table 4) (Fig. 5). By using ^{14}C AMS analysis of SOM, Krull *et al*. (2005) demonstrated in a similar study that the POC pool in the uppermost 30 cm is generally composed of young and labile material with mean residence times of around 50 years for the >200-μm fraction and around 80 years for the 53–200-μm fraction. By comparison, mean residence time of the bulk SOM in the uppermost 30 cm was about 400 years. Thus, the >200-μm fraction tended to be more sensitive to short-term, cyclical vegetation changes (e.g. because of drought or fire) than the 53–200-μm fraction. In this study, the occurrence of such short-term cycles is suggested by the variation of $\delta^{13}C$ values in the >200-μm fraction in both sites. The relative stability of a system (i.e. whether a system is affected by recent or ongoing vegetation change) can be assessed by the differences between the $\delta^{13}C$ values of the POC fraction and those of the whole soil, as shown by Krull *et al*. (2005). Large differences (>1.5‰) between the POC fractions and the whole soil are interpreted as an indicator for a disequilibrium between the contemporary system, represented by the ~50–100-year-old POC pool, and the long-term soil organic carbon pool (represented by the whole soil) (Krull *et al*. 2005). The isotopic difference between the 53–200-μm fraction and the whole soil for the Tree site was ±0‰ in the 0–5 cm and 2.4 ± 0.4‰ in the deeper soil profile. By comparison, the isotopic difference for the Open site averaged 0.5‰ throughout the soil profile. These data indicate that the Tree site must have attained its present woody state fairly recently (<100 years), as the C3-dominated 53–200-μm fractions differed significantly from the whole soil $\delta^{13}C$ values below 5 cm. By comparison, the lack of a significant difference between the 53–200-μm fraction and the whole soil from the Open site point towards a much greater stability with respect to vegetation composition over the last 100 years.

The ^{13}C-enrichment in SOM from the surface to 30 cm depth was 4.5‰ at the Tree and 2.8‰ at the Open site. While the ^{13}C-enrichment with depth at the Tree site (>3‰) can be attributed to some degree to vegetation change from less to more woody biomass (Balesdent *et al*. 1993; Boutton *et al*. 1998; Biggs *et al*. 2002), the 2.8‰ ^{13}C-enrichment in the Open site in the uppermost 30 cm is difficult to attribute to either changes in C3–C4 proportion or to ascribe to fractionation during decomposition. This inherent (unrelated to any C4 input) increase in $\delta^{13}C$ values in SOM could be at least partly attributed to a proportionally greater input of ^{13}C-enriched root material than ^{13}C-enriched litter material (Table 1) with increasing soil depth (Bird *et al*. 2003). However, $\delta^{13}C$ values of humified and older SOM are commonly ^{13}C-enriched compared with litter material, suggesting that fractionation during decomposition determines to a large extent $\delta^{13}C$ changes with depth (Balesdent and Mariotti 1996; Boutton 1996). It is possible to estimate the likely $\delta^{13}C$ change due to fractionation associated with decomposition processes by applying an approximation of the Rayleigh equation, as used by Mariotti *et al*. (1981), Balesdent and Mariotti (1996), Krull *et al*. (2002) and Accoe *et al*. (2003), and discussed in detail by Wynn *et al*. (2005):

$$\delta^{13}C_{\varepsilon} = ((\mathrm{Ln}\,(OC_I/OC_S)) \times \varepsilon) + \delta^{13}C_S, \qquad (3)$$

where $\delta^{13}C_{\varepsilon}$ is the calculated $\delta^{13}C$ value by using the Rayleigh enrichment factor, OC_I is the measured OC content at each depth increment, OC_S is the OC content in the 0–2-cm interval, ε is the Rayleigh factor and $\delta^{13}C_S$ is the $\delta^{13}C$ value at the surface. Balesdent and Mariotti (1996) empirically determined a value for ε of −1.71, whereas Accoe *et al*. (2003) reported ε values ranging from −1.6 to −1.9, depending on the clay content of the soil. These studies indicate that the value of ε is site-specific and should be determined independently. We utilised the $\delta^{13}C$ depth trend

of the Open site to determine the value for ε, assuming that the $\delta^{13}C$ changes at this site are mainly governed by fractionation during decomposition. Apart from the 50–70-cm interval, an ε value of −1.6 best approximated the $\delta^{13}C$ depth trend of the Open site, which is in good agreement with the value of −1.64 for loamy sand soils, determined by Accoe *et al*. (2003).

The equation uses $\delta^{13}C_S$ as the initial value, and isotopic changes are calculated as a function of the changes in carbon content with depth (OC_I/OC_S). Figure 6 shows the measured $\delta^{13}C$ data for the Open and Tree site and the calculated $\delta^{13}C$ values, by using Eqn 3. Owing to the very different litter and surface $\delta^{13}C$ values at the Open site, we calculated the enrichment trends for two different scenarios for both sites: (1) initial value = surface soil ($\delta^{13}C\ R_S$) and (2) initial value = litter ($\delta^{13}C\ R_L$). The data show that in the Tree site the results from the two scenarios were virtually the same and that the estimated ^{13}C-enrichment due to fractionation associated with decomposition in the uppermost 30 cm was 3.0‰. The fact that the measured $\delta^{13}C$ difference was an additional 1.5‰ greater supports the interpretation that the measured $\delta^{13}C$ trend under the Tree site was due to progressively greater tree cover and a greater proportion of C3-derived carbon in the soil carbon pool. By comparison, results from the application of Eqn 1 without the necessary correction for fractionation during decomposition suggest an apparent change from the proportion of C3-derived carbon from 25% (at depth) to 60% (soil surface). Taking into consideration the $\delta^{13}C$ change from decomposition (Eqn 3), results from Eqn 1 overestimate the C3-derived contribution by close to 10% in the 5–10-cm interval and by 15% at greater depths. Similar principles apply to the Open site, where Eqn 1 estimates a change in C3-derived proportions from 10% (at depth) to 30% (at the soil surface), yet the $\delta^{13}C$ trend with depth can be approximated very closely by application of the Raleigh factor ε without any changes in the C3/C4 proportions.

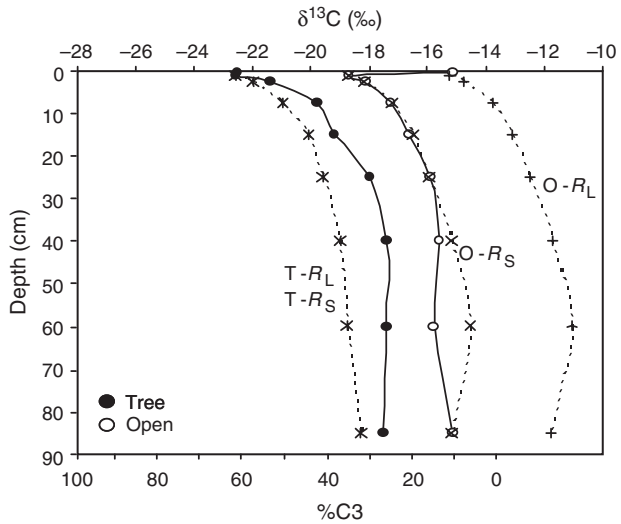

Fig. 6. $\delta^{13}C$ values of whole SOM from the Tree and Open sites as in Fig. 5 and the calculated $\delta^{13}C$ depth trends using Eqn 3, where $\delta^{13}C_M$ = measured $\delta^{13}C$, $\delta^{13}C_{R-S}$ = calculated $\delta^{13}C$ (using the 0–2 cm measured $\delta^{13}C$ as initial value) and $\delta^{13}C_{R-L}$ = calculated $\delta^{13}C$ (using measured $\delta^{13}C$ from litter as initial value). The lower *x*-axis shows the proportion of C3-derived carbon from Eqn 1 and without correction for fractionation from decomposition.

While the two scenarios for the Open site had the same enrichment value of 2.7‰ from the surface to 30 cm depth, the absolute $\delta^{13}C$ trends with depth were significantly different. The results from Scenario 1 (using the surface soil $\delta^{13}C$ value as the starting point) matched the measured data, suggesting that the litter $\delta^{13}C$ values and the open, grassy vegetation state are not representative of the average, longer-term vegetation history. Accordingly, the present vegetation state must have been attained very recently and over a time frame that was short enough to preclude the litter-derived $\delta^{13}C$ signal to be included in SOM. Otherwise, if the current vegetation state was representative of the average, longer-term vegetation history, the depth trend represented by Scenario 2 would have been more closely matched with the measured data.

On the basis of these observations, the current vegetation composition of the Open site appears to be the result of a very recent change towards a greater proportion of grassy vegetation and is therefore not representative of the average vegetation history of the site (represented by the $\delta^{13}C$ value of the surface SOM).

Such recent changes cannot be resolved by ^{14}C analysis, as the ^{14}C activity in SOM cannot usually successfully resolve changes within the last 40 years due to SOM being composed of different organic carbon pools with widely ranging turnover times (e.g. Wang *et al*. 1996; Trumbore 2000). Comparison of the $\delta^{13}C$ record with aerial photography could help to verify whether the Open site recently had a greater proportion of grasses. Figure 7 illustrates the relative changes in tree cover at the Tree and Open site, using the earliest available aerial photograph (1951) as the baseline (100%). Any changes in tree cover would consequently deviate above or below the '100' baseline value. Aerial cover of the

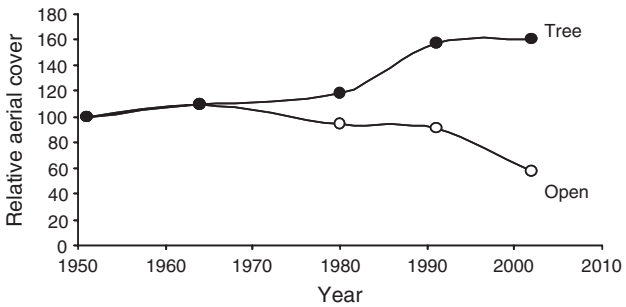

Fig. 7. Relative tree cover estimated from aerial photographs for the Tree and Open sites since 1951.

Open site remained stable until 1990, after which tree cover declined by about 40% relative to the 1950s. By comparison, aerial cover at the Tree site showed a relative increase from the late 1970s onwards. Droughts during the mid 1980s and early 1990s had affected the Open site to a greater degree than the Tree site and were probably responsible for the digression between surface soil and litter $\delta^{13}C$ values. This interpretation of the aerial photographs is supported by the tree size class distributions and oral history for the two sites (Fig. 1).

Conclusion

Two sites (Open and Tree) in north-eastern Queensland were studied with respect to recent and longer-term vegetation change histories. We assessed the degree of variability imparted by these mixed tree–grass systems on soil texture and organic carbon data. We found that the statistical error was relatively small for variations in inorganic properties (%clay) of clay and TOC contents. By comparison, $\delta^{13}C$ values of soil and litter showed much larger standard deviations, particularly in the soil surface and deepest horizons, attesting to the spatial and temporal variability in carbon input into the soil. Results from $\delta^{13}C$ analyses of whole soil and POC fractions showed that the Tree site recorded a recent trend towards a greater proportion of woody biomass (based on published turnover times of soil POC fractions). Within this overall trend towards greater woody biomass, short-term cyclical vegetation changes, most likely due to drought or fire, were evident in the $>$200-μm fractions. By comparison, the $\delta^{13}C$ values of whole soil and POC fractions of the Open site indicated that this site had remained relatively stable over the last 100 years or longer. After correction of the $\delta^{13}C$ values for fractionation due to decomposition, the apparent enrichment with depth at the Open site was not large enough to account for any significant vegetation changes during the longer-term history. By comparison, the ^{13}C-enrichment at the Tree site could be attributed to a change from a more open to a more woody system. Thus, the isotopic record from the SOM, which allows for a resolution of $>$50 years, indicates an on average stable Open site and a 'thickening' Tree site. However, the large shift observed in the $\delta^{13}C$ values of the litter material at the Open site points towards a shift that was too recent ($\sim$10 years) to be incorporated in the SOM pools and therefore was not apparent in the soil $\delta^{13}C$ values. Aerial photographs showed that, compared with the Tree site, the Open site had failed to recover from the severe droughts in the mid 1980s and early 1990s, resulting in tree dieback and change towards a more open state. Thus, our data indicate that isotopic values of soil, litter and plant material can be used to reconstruct short, intermediate and long-term vegetation changes in areas where there is a clear distinction between photosynthetic pathways between grasses and trees.

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