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ORIGINAL ARTICLE

Stable forest–savanna mosaic in north-western Tanzania: local-scale evidence from $\delta^{13}C$ signatures and ^{14}C ages of soil fractions

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ABSTRACT

Aim The spatio-temporal dynamics of dry evergreen forest patches in the savanna biome of the Kagera region (north-western Tanzania) are largely unknown owing to a lack of pollen and macrofossil evidence. Our aims were to reconstruct local-scale shifts of the forest–savanna boundary in order to determine whether the forests have been expanding or retreating on a centennial and millennial time-scale.

Location The Kagera region of north-western Tanzania, East Africa.

Methods The vegetation reconstruction was based on analysing $\delta^{13}C$ signatures in soils along a transect spanning both C_4 open savanna and C_3 forest vegetation. Furthermore, we fractionated soil organic matter (SOM) according to density and chemical stability to analyse $\delta^{13}C$ values of soil fractions with distinct radiocarbon ages.

Results We found sharp changes in $\delta^{13}C$ signatures in bulk SOM from the forest to the savanna, within a few metres along the transect. The forest soil profiles carried a persistent C_3-dominated signature. Radiocarbon dating of the oldest, most recalcitrant forest soil fraction yielded a mean age of 5500 cal. yr BP, demonstrating that the forest has existed since at least the mid-Holocene. The savanna sites showed a typical C_4 isotopic signature in SOM of topsoils, but subsoils and more recalcitrant SOM fractions also contained signals of C_3 plants. The dense soil fraction ($\rho > 1.6$ g cm^{-3}) carrying a pure C_4 label had a mean age of *c.* 1200 cal. yr BP, indicating the minimum duration of the dominance of grass vegetation on the savanna site. At the forest edge, the older C_4 grass signature of SOM has steadily been replaced by the more negative $\delta^{13}C$ fingerprint of the forest trees. As this replacement has occurred mainly in the 10-m-wide forest–savanna ecotone over the last *c.* 1200 years, the forest expansion must be very slow and is very likely less than 15 m century^{-1}.

Main conclusions Our results suggest that forest patches in the Kagera savanna landscape are very stable vegetation formations which have persisted for millennia. During the last millennium, they have been expanding very slowly into the surrounding savanna at a rate of less than 15 m century^{-1}.

Keywords
^{13}C, C_3 plants, C_4 plants, dry evergreen forest, East Africa, ecosystem dynamics, savanna, SOM fractionation, vegetation mosaic, vegetation reconstruction.

INTRODUCTION

The savanna biome is of increasing interest to scientists because it covers more than 20% of the Earth's terrestrial surface and accommodates a fifth of the human population, mostly in less-developed countries (Mistry, 2000). Ongoing climate change and increasing human pressure mean that the biodiversity and ecological functioning of the savannas is

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potentially at risk (e.g. Vincens *et al.*, 1999; Asner *et al.*, 2009). Moreover, people living on local resources are exposed to greater economic vulnerability (e.g. Higgins *et al.*, 1999). Therefore, a deeper understanding of the history, nature and dynamics of the savanna biome is crucial in order to mitigate future trends (Scholes & Archer, 1997) and to promote the sustainable management of these landscapes.

The dynamics of savanna landscapes in East Africa are controversial. Troupin (1966) and Rodgers *et al.* (1977) postulated that thicket clumps and small patches of forest on stony hillsides are relicts of previously large forests. However, in many tropical dry ecosystems, woody plant abundance has substantially increased in recent history (Kendall, 1969; Vincens *et al.*, 1999; Gillson, 2004; Durigan & Ratter, 2006). The expansion of woody formations may be driven by several factors: increased rainfall (e.g. Ratter, 1992 for Brazil, or Schwartz *et al.*, 1996 for Congo), reduced herbivore browsing (e.g. Asner *et al.*, 2009 for South Africa), a change in fire regime (e.g. Bowman *et al.*, 2008 for Australia), termite activities (e.g. Pullan, 1979 for southern Africa), atmospheric CO_2 enrichment (e.g. Kgope *et al.*, 2010), or a combination of these factors (e.g. Roques *et al.*, 2001 for Swaziland, or Bloesch, 2002, 2008 for East Africa). It is worth noting that the expansion of forest is often masked by forest clearing, mainly for logging and agricultural purposes.

Although these vegetation shifts have been widely reported, the rates and patterns of these changes have seldom been quantified on decadal, centennial or even millennial time-scales. Environmental conditions within savanna landscapes with alternating dry and wet seasons often preclude the formation of permanent lakes and bogs, which could provide a continuous long-term pollen record. Thus, reconstruction of East African vegetation is mainly based on terrestrial palynological studies from high elevations, sediment cores from Lake Victoria and some plant macrofossil records (e.g. Kendall, 1969; Jolly *et al.*, 1998; Elenga *et al.*, 2000). Moreover, pollen profiles barely reflect recent changes over the last centuries or decades and they integrate over large areas and across vegetation boundaries (Gillson, 2004), and are thus inappropriate for reconstructing small-scale vegetation changes.

Alternatively, past vegetation changes in the savanna landscape can be reconstructed on a local scale using stable carbon isotope ($\delta^{13}C$) analysis in soils. Most tropical grasses and sedges utilize the C_4 photosynthetic pathway, and are enriched in ^{13}C ($\delta^{13}C = -11$ to $-13‰$) compared with trees, shrubs and forbs, which utilize the C_3 photosynthetic pathway and discriminate more strongly against ^{13}C in the atmosphere ($\delta^{13}C = -26$ to $-29‰$) (Fry, 2006). These large differences in $\delta^{13}C$ are almost entirely preserved in soil organic matter (SOM), and they thus allow the estimation of site-specific vegetation changes (Schwartz *et al.*, 1996; Gillson, 2004), which is particularly useful for the assessment of vegetation boundary shifts. However, SOM consists of a continuum of younger and older components. Therefore, even when the $\delta^{13}C$ measurements at different soil depths are combined with radiocarbon-based estimates of mean SOM ages, the temporal resolution of past vegetation changes is low (e.g. Boutton *et al.*, 1998). One approach to overcome this shortcoming is to separate SOM fractions with a delimited age. Separation of soils according to their density, particle size or after chemical oxidation has been found to yield SOM fractions with distinctly different turnover rates, and thus distinct and relatively delimited ages (Christensen, 1992; Krull *et al.*, 2005; Mikutta *et al.*, 2005; Favilli *et al.*, 2008).

In our study we assessed the spatio-temporal dynamics between the forest patches and the open savanna in the Kagera region of north-western Tanzania. Our approach involved measuring $\delta^{13}C$ signatures in SOM along a transect from an isolated, dry evergreen forest – the Kagoma Forest – to the currently surrounding open savanna. Furthermore, we also analysed stable isotopes in and determined radiocarbon ages of SOM fractions that were separated according to a particular set of physical (density) and chemical fractionation techniques that yield organic matter of defined ages in these tropical soils. Our aim was to determine how stable the forest patches are over time and whether the forests have been expanding or retreating on a centennial and millennial time-scale.

MATERIALS AND METHODS

Study area

The study area, the Kagoma Forest and its immediate vicinity, is situated in the eastern part of the Kagera region, in the Bukoba district of Tanzania (1°36′3″ S, 31°18′2″ E), close to the Rwandan and Ugandan borders (Fig. 1, left). The landscape is hilly and lies between 1100 and 1800 m a.s.l. Shales, sandstones and other mostly terrestrial sedimentary rocks of the Bukoban System constitute the bedrock in the eastern Kagera. Phyllites and low-grade schists are prevalent in the more metamorphosed western part (Schlüter, 2006). The geological parent material generally leads to deeply weathered, argillic soils at the valley bottoms and in gullies. These dark-brown clay loam profiles are classified as Nitisols (Bloesch, 2002; FAO, 2006). On the stony hillsides, however, less developed soils are normally encountered, with only a shallow umbric horizon over brittle, frequently red coloured, shales, which are defined as dystric Leptosols (Bloesch, 2002; FAO, 2006). The mean annual temperature is about 21 °C, with diurnal cycles that reach from 12 to 34 °C (Bloesch, 2008). The rainfall regime is bimodal, peaking in March–May and to a lesser extent, in October–December, while the main dry season lasts about 100 days, from May until August (Troupin, 1966). The total annual precipitation ranges from 575 mm to about 1300 mm, and is quite unpredictable and irregular in time and space (Bloesch, 2002). The landscape is dominated by open savanna vegetation, whereas a few dense forest patches can be found within and around gullies. These dry evergreen forests are typically small (a few square kilometres). Appendix S1 in Supporting Information gives a list of typical plant species, their respective cover-abundance and an overview of the sharp vegetation change between the forest and the surrounding

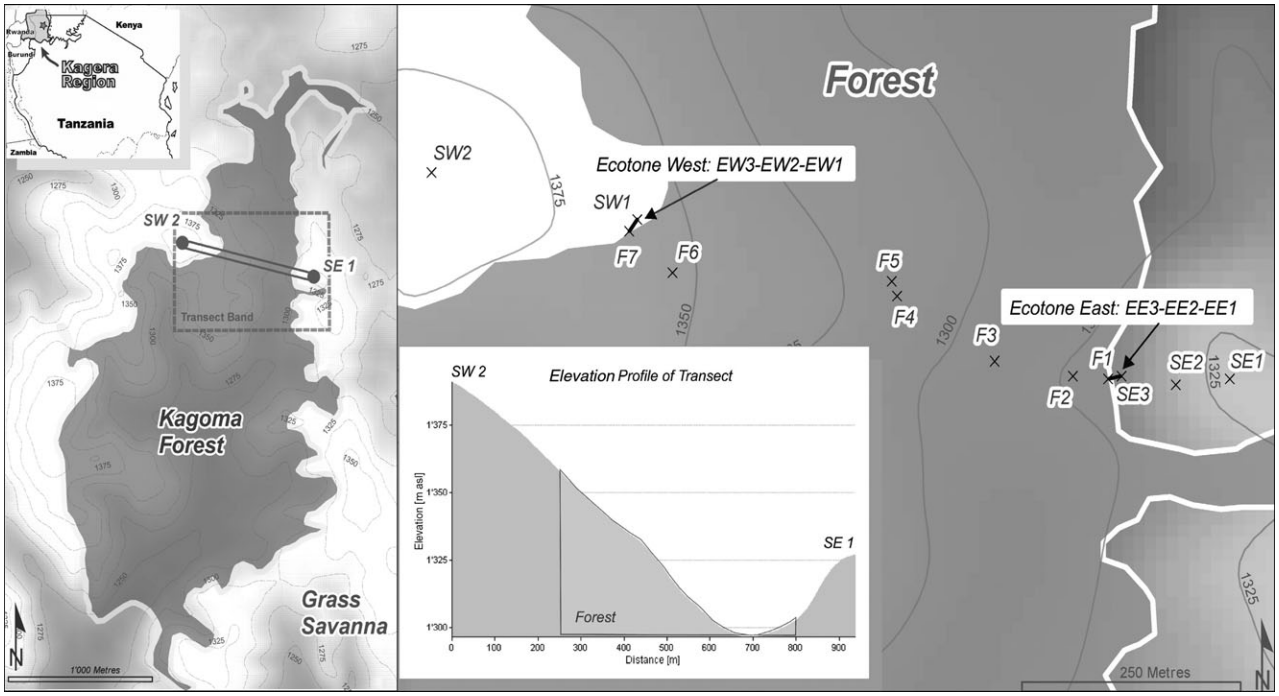

Figure 1 Left: Overview map of the Kagoma Forest in north-western Tanzania (1°36′36″ S, 31°18′20″ E) and the transect band that was chosen for the sample collection. Right: Close-up view of the transect band and the individual sites. Note the narrow and sharp ecotone between the forest and the open savanna. (Maps were created on the basis of Landsat 7 Global Land Survey red and middle infrared channels and the SRTM90 dataset.)

savanna. Direct human impact can still be assumed to be low at the study site.

Plant and soil sampling

We excavated 18 soil profiles along an east–west transect, approximately 950 m in length, across the Kagoma Forest (Fig. 1). The profile sites were sampled irregularly along the transect but in defined strata (strata: savanna east 'SE', ecotone east 'EE', forest 'F', ecotone west 'EW', savanna west 'SW'). Using these strata allowed us to take into account small-scale heterogeneities. The stratification also helped to obtain a higher spatial resolution at the potentially most interesting sites, the two edges of the forest, where we sampled three ecotone profiles (EE1, EE2, EE3 and EW1, EW2, EW3) within 10 m distance in each case.

The 18 profiles were systematically sampled at the following depth intervals: 0–2, 2–5, 5–10, 10–30, 30–50, 50–70, 70–100 cm. Samples were sieved to < 2 mm and dried at 50 °C. As all samples had pH < 4.0 ($CaCl_2$), removal of carbonate prior to carbon measurements was not necessary. In addition to soils, we also sampled litter and biomass from both vegetation types and the ecotone. Lithological material was taken at site EE2 (60 cm depth). We dried the litter and biomass samples at 50 °C for at least 96 h, and then ground them with a ball mill. The stone sample had first to be crushed with a clean hammer and anvil before it was finely ground as well.

Soil fractionation procedure

Based on the results of the stable isotope analysis of bulk soils, fractionation of soils was only carried out on a subset of the original 18 soil profiles: (1) the site SE1 and the two sites F1 and F3 to represent the open savanna and forest ecosystem, respectively; (2) the ecotone profiles EE1, EE2 and EW3 at the forest edge, which exhibited an irregular bulk isotopic depth gradient and thus a changing contribution of C_3 and C_4 plants. For the soil fractionation procedure (Fig. 2), we separated the bulk soil according to its density into a free light fraction ('soilLF'), occluded light fraction ('oLF') and a dense fraction ('DF'). The dense fraction was then chemically treated with alkali–acid solutions (to yield the 'huminDF') and hydrogen peroxide (which yielded the 'recalcitrantDF').

Density fractionation

First, we isolated the light fraction ('LF') out of the bulk soil samples by slightly shaking the soil material in a polytungstate solution (10%, wt/wt), which was adjusted to a density of 1.6 g cm^{-3} (Kaiser & Guggenberger, 2007). Any charred fragments in this light fraction, the 'charLF', were separated by hand from the LF under a stereo microscope (Leica Wild M3Z, Wetzlar, Germany) because they slow down the mean turnover time of the fraction significantly (Murage *et al.*, 2007). The char-free material was named 'soilLF'. The residual sample material (the non-LF) was then ultrasonically dispersed in the density

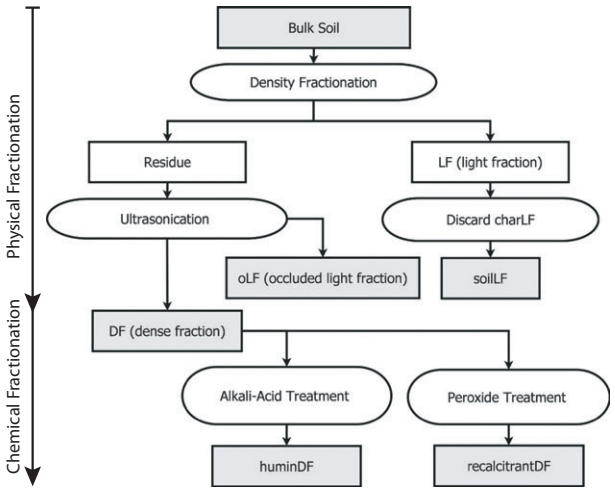

Figure 2 Flow chart that shows the fractionation scheme. Boxes highlighted in grey represent sample sets that were examined for ^{13}C isotopic content ($\delta^{13}C$) by mass spectrometry.

solution by applying an energy of 450 J mL^{-1} (calorimetrically calibrated according to Roscoe *et al.*, 2000) using a Bandelin HD3200 device (Berlin, Germany). This effectively disintegrates aggregates into primary organomineral complexes without causing methodological artefacts (Schmidt *et al.*, 1999). After the ultrasonic treatment, the occluded light fraction ('oLF') could be isolated from the sedimented dense fraction ('DF').

Alkali–acid treatment of DF

Adopting the alkali–acid procedure of Rethemeyer *et al.* (2005), we added 20 mL of 1% NaOH to 3.0 g of sample material in a container and placed it into a shaking waterbath at a temperature of 60 °C for 4 h. The non-soluble residue was then washed with ultrapure water until a pH of 10 was reached. Afterwards, the substance was treated with 30 mL of 1% HCl for 10 h to remove any atmospheric CO_2 that might have been introduced during the alkali treatment. The resulting fraction, the 'huminDF', was finally rinsed with water to pH > 4, and then dried in the oven at 50 °C.

Hydrogen peroxide treatment of DF

In order to get the oldest, most recalcitrant SOM fraction, an oxidation treatment with hydrogen peroxide is recommended (Mikutta *et al.*, 2005). We closely followed the procedure of Favilli *et al.* (2008), but used twice the amount of sample material. In short, 2.0 g from each sample was wetted in a beaker glass for 10 min using ultrapure water. Then, 180 mL of 10% H_2O_2 was added and the container was sealed with two layers of Parafilm to avoid evaporation of the reagent. A magnetic stirrer hotplate constantly mixed and heated the peroxide suspension to 50 °C during the whole treatment (96 h). Afterwards, the samples were washed three times with 80 mL of Millipore water and freeze-dried.

Stable isotope analyses

Aliquots of plants, litter, bulk soils and all soil fractions were weighed into ultra-clean tin capsules and sealed. They were combusted and analysed for $\delta^{13}C$ and carbon contents on a Thermo IR-MS Euro Elemental Analyzer (Bremen, Germany). Isotope results are reported in conventional δ notation as per mil (‰) relative to the carbon isotopic ratio of the Vienna PeeDee Belemnite (VPDB) standard (Fry, 2006). Absolute measurement uncertainty was ± 0.2‰ for $\delta^{13}C$ and the relative uncertainty was ± 10% for carbon content.

Subsequent statistical analyses and tests were performed with SPSS 16.0.2 (Chicago, IL, USA).

Radiocarbon dating

Samples of isolated soil fractions from a soil profile in the forest (F3) and the savanna (SE1) were graphitized and analysed at the accelerator mass spectrometry (AMS) facility of the ETH-PSI, Zürich. Results were corrected for blank values and isotopic fractionation. Conventional radiocarbon ages were calculated following the convention of Stuiver & Pollach (1977) and calibrated using the OxCal program and the ShCal04 calibration curve (Bronk Ramsey, 2001).

Bulk soil ^{13}C depth enrichment due to humification

The Rayleigh enrichment factor ε describes the increase in $\delta^{13}C$ with profile depth in bulk soil. This increase is due to isotopic fractionation during the decomposition processes of organic matter (humification) and is unrelated to vegetation change. The factor ε was estimated by applying an approximation of the Rayleigh equation (Balesdent & Mariotti, 1996; Wynn *et al.*, 2005):

$$\delta^{13}C_{\varepsilon} = \{[\ln(OC_I/OC_S)] \times \varepsilon\} + \delta^{13}C_S \qquad (1)$$

where $\delta^{13}C_{\varepsilon}$ is the measured carbon isotopic signature for each depth increment I with a measured organic carbon (OC) content of OC_I, and $\delta^{13}C_S$ and OC_S represent the initial values from the soil surface S (0–2 cm depth). The factor ε for each profile was estimated by fitting equation 1 to the measured data by the least squares method.

The above model was used to distinguish the pedogenetically induced depth enrichment in ^{13}C (due to humification) from other isotopic effects (e.g. due to vegetation change). It is only applicable to bulk soil profiles.

RESULTS

$\delta^{13}C$ signatures in bulk soils along the transect

Soil organic carbon (SOC) contents were smaller in the savanna than in the forest (Fig. 3). SOC strongly declined with depth in all soils and reached rather low values of mostly less than 1% SOC, especially in the subsoils of the open savanna ecosystem.

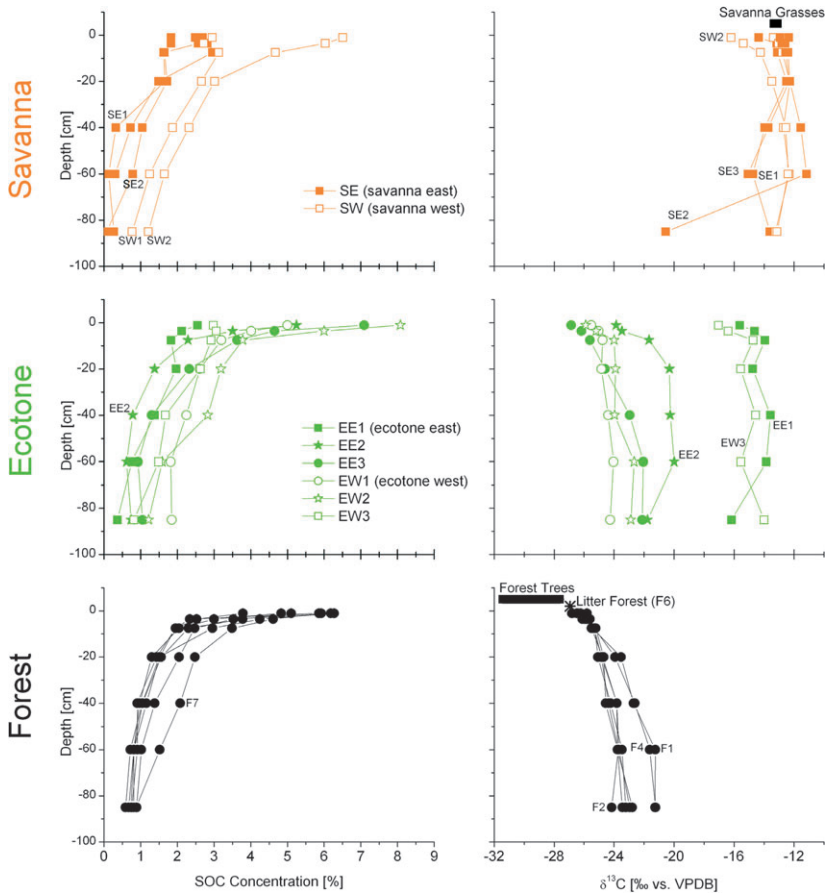

Figure 3 The bulk soil results display characteristic traits of the three ecosystems (savanna, ecotone and forest) studied in north-western Tanzania with respect to carbon concentration and carbon isotopic composition.

Along the transect, there was a sharp change in $\delta^{13}C$ signatures from the savanna across the Kagoma Forest (Fig. 3 and Appendix S2). The three vegetation strata along the transect (forest, ecotone, savanna) featured significantly different $\delta^{13}C$ values in SOM ($P < 0.001$, Kruskal–Wallis test), with the $\delta^{13}C$ ratios decreasing from the savanna to the ecotone, and even more in the forest soils.

In the forest and the savanna, the carbon isotopic signature of the uppermost horizons corresponded closely to the measured isotopic signature of the vegetation cover (data not shown). In the forest soil profiles, the $\delta^{13}C$ values increased linearly with decreasing ln(OC). In the savanna profiles, the $\delta^{13}C$ values showed a curved relationship to ln(OC), with the $\delta^{13}C$ values decreasing with lower OC contents in the subsoils (Fig. 4). Within the 10-m wide forest–savanna ecotone in the east and west, the $\delta^{13}C$ values changed from almost typical forest profiles to savanna profiles (e.g. EE1 to EE3, Fig. 4). These sites at the current forest edge constituted an intermediate state between the typical savanna and the forest $\delta^{13}C$ profiles. For example, the ecotone profiles towards the savanna on the eastern (EE1) and western (EW3) side of the transect resembled the savanna soils (Fig. 3), but were significantly depleted in ^{13}C ($P = 0.006$, Mann–Whitney U-test). The transitional stage of the ecotone profiles was further supported by their more pronounced variation in $\delta^{13}C$ values (Fig. 3).

$\delta^{13}C$ and ^{14}C signatures of soil fractions

Density fractionation clearly separated particulate organic matter, with organic carbon (OC) contents above 33% in the two light fractions (soilLF, oLF) and below 2% in the DF, the dense, mineral-associated fraction (Table 1). The light fractions comprised 5–35% of the total bulk SOC. Thus, despite its low OC concentration, the DF stored the major part of total SOC, even in the uppermost soil horizons. The alkali–acid and peroxide treatment removed significant amounts of SOC from the DF. The lowest OC concentrations were found in the recalcitrant DF, which had less than 7% of the initial bulk SOC contents.

The fractions showed consistent $\delta^{13}C$ signatures across the different sampling depths of a profile (cf. the small error bars in Fig. 5). This again indicated that our fractionation procedure can isolate relatively homogeneous fractions. Typical current plant inputs within each ecosystem were reflected by the $\delta^{13}C$ value of the free light fraction (soilLF), and similarly by the occluded light fraction (oLF), while the DF was

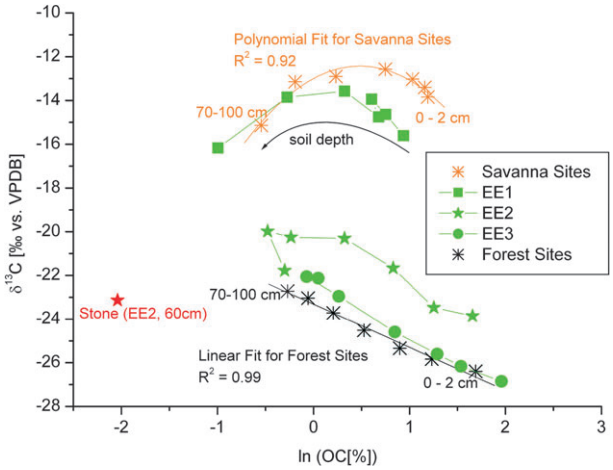

Figure 4 The relationship between the organic carbon (OC) concentrations and $\delta^{13}C$ values of the bulk soil profiles from north-western Tanzania. The savanna graph shows the means of all five savanna profiles (SE1–3, SW1–2), and the forest graph of all seven forest profiles (F1–F7). The eastern ecotone profiles (EE1, EE2, EE3) show the rapid transition at the forest edge: the $\delta^{13}C$ signature of the three profiles changes completely within 10 m. Isotope results are reported in conventional δ notation as per mil (‰) relative to the carbon isotopic ratio of the VPDB standard (Fry, 2006).

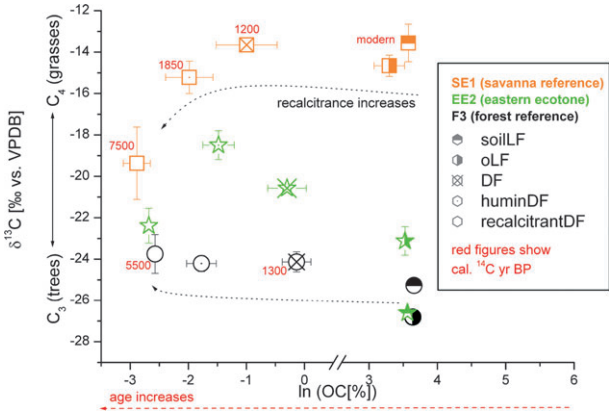

Figure 5 The relationship between the organic carbon (OC) concentrations and the $\delta^{13}C$ values of soil fractions is displayed for three selected profiles from north-western Tanzania (symbols show the average across all profile depths). The mean ^{14}C age (and therefore recalcitrance) increases with decreasing OC concentrations. The data from the western side of the forest–grass savanna transition (with profile EW3 instead of EE2) look the same (data not shown). soilLF, free light fraction; oLF, occluded light fraction; DF, dense fraction. Isotope results are reported in conventional δ notation as per mil (‰) relative to the carbon isotopic ratio of the VPDB standard (Fry, 2006).

representative of the bulk soil $\delta^{13}C$ signature due to its high contribution to total SOC. The chemically treated DF had approximately the same $\delta^{13}C$ values as the bulk soil in the forest, but showed deviating $\delta^{13}C$ values in the ecotone and in the savanna.

The radiocarbon dating of soil fractions (see Appendix S3) clearly showed that the combined density-chemical fractionation separated SOM pools of distinct ages (Fig. 5). The light fraction consisted of 'modern' OC that had been assimilated after the nuclear bomb testing in the 1950s. In both the savanna and the forest soils, the DF had a mean carbon residence time of the order of 1200 and 1300 years, respectively. Chemical removal of SOC from the dense fraction further increased the mean ^{14}C ages. After treatment with hydrogen peroxide, the soil residues had a calibrated ^{14}C age of 5500 years in the forest and of 7500 years in the savanna, respectively.

DISCUSSION

On a local scale, past vegetation changes have been assessed by analysing the $\delta^{13}C$ signatures from savanna grasses or from trees in bulk SOM (e.g. Schwartz *et al.*, 1996; Gillson, 2004). But because bulk SOM comprises components of different ages, the time-scale of vegetation changes remains uncertain even when radiocarbon ages are determined. Our fractionation of SOM according to soil density and chemical stability

Table 1 Mean organic carbon (OC) concentrations of the soil fractions from the savanna, ecotone and forest sites, which are located in north-western Tanzania. The data for the fractions are based on the fractionated samples from SE1, EE1, EE2, EW3, F1 and F3 for the savanna, ecotone and forest, respectively. Values are the mean of *n* samples.

		Soil fractions											
		Bulk soil			soilLF			oLF		DF		huminDF	recalcitrantDF
	Depth (cm)	*n*	Mass (%)	OC (%)	*n*	Mass (%)	OC (%)	Mass (%)	OC (%)	Mass (%)	OC (%)	OC (%)	OC (%)
Savanna	0–30	20	100	2.85	2	1.13	33.09	0.28	43.66	98.59	1.25	0.35	0.10
	30–100	15	100	0.91	3	0.10	38.67	0.07	20.31	99.84	0.18	0.08	0.05
Ecotone	0–30	24	100	3.59	6	1.20	38.79	0.52	42.30	98.29	1.71	0.43	0.10
	30–100	18	100	1.31	9	0.42	40.02	0.15	31.81	99.44	0.70	0.18	0.08
Forest	0–30	28	100	3.25	4	1.37	33.05	0.78	43.02	97.85	1.76	0.38	0.12
	30–100	21	100	0.98	6	0.25	37.44	0.16	36.96	99.59	0.58	0.12	0.07

soilLF, free light fraction; oLF, occluded light fraction; DF, dense fraction; OC, organic carbon.

improved the timeline for vegetation reconstruction by separating SOM fractions of distinct ages, spanning from young ('modern'), i.e. less than 50-year-old carbon in the light fraction to 7500-year-old carbon in the most recalcitrant fraction (Fig. 5).

Persistent forest vegetation at forest sites

The $\delta^{13}C$ and ^{14}C signatures along the savanna–forest–savanna transect and between soil fractions showed that the forest–savanna boundary has been rather stable during the last millennium and that the forest has been very slowly expanding into the savanna. The high temporal persistence of the forest patch was indicated by a continuously C_3-dominated signature in the bulk SOM of the forest profiles (Figs 3 & 4). Although the bulk SOM in the subsoils of the forest showed 4–5‰ higher $\delta^{13}C$ values than the topsoils, it seems likely that this depth enrichment is not due to a former C_4 vegetation input. Rather, it can be explained by isotopic fractionation processes during humification (Wynn *et al.*, 2005). The modelled increase in $\delta^{13}C$ values with decreasing OC contents using the approximation of the Rayleigh equation (equation 1) yielded an enrichment factor ε of −1.82‰ (Fig. 6). This value is in good agreement with the ε factor of fine-textured soils, which typically ranges between −1.6 and −1.9‰ (Accoe *et al.*, 2003; Krull & Bray, 2005). We therefore conclude that the observed increase in the $\delta^{13}C$ value with depth can largely be attributed to the isotopic fractionation during humification in the forest soils and not to a greater contribution of C_4 grasses in the past. Our results from the SOM fractionation procedure support this conclusion. None of the isolated fractions of the forest soils, including even the most recalcitrant (i.e. oldest) ones, showed any signs of savanna vegetation in the past (Fig. 5). Radiocarbon data indicated a mean carbon residence time of 5500 years in the recalcitrant fraction of the forest, which demonstrates that the forest has existed since at least mid-Holocene times.

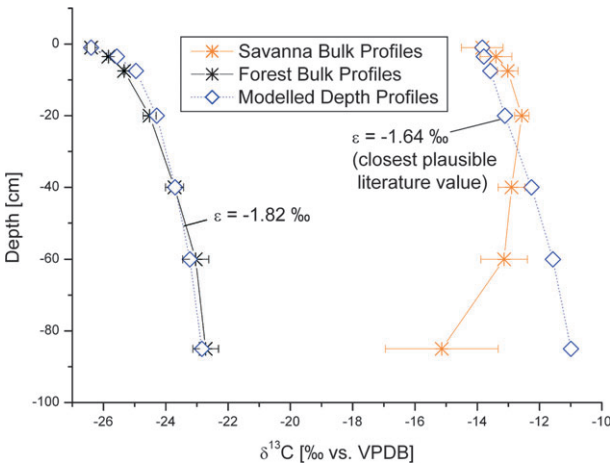

Figure 6 Measured and modelled depth enrichment due to decomposition processes (humification). The modelled savanna depth profile was calculated with a value of ε = −1.64‰ (derived from Accoe *et al.*, 2003), because the fitted enrichment factors were not plausible. The savanna graph shows the mean of all five savanna profiles (SE1–3, SW1–2) and the forest graph of all seven forest profiles (F1–F7). Isotope results are reported in conventional δ notation as per mil (‰) relative to the carbon isotopic ratio of the VPDB standard (Fry, 2006). The study area is located in north-western Tanzania.

Persistent savanna vegetation at savanna sites

The $\delta^{13}C$ signatures in both the bulk soil profiles and the soil fractions indicate a changing contribution of C_3 and C_4 plants to SOM of the currently open savanna 100 m from the forest edge. In the uppermost 30 cm, the enrichment of the savanna soils in ^{13}C agrees with the isotopic fractionation according to the Rayleigh model, but strongly deviates from it in the deeper soil horizons (Fig. 6). The $\delta^{13}C$ signatures in the isolated SOM fractions show the same pattern: the humin and the recalcitrant fraction are depleted in ^{13}C as compared with the younger fractions (Fig. 5). As isotopic fractionation during humification would lead to the opposite trend, i.e. to a ^{13}C enrichment of the older fractions, our findings clearly indicate a greater contribution of C_3-plants to SOM in the deeper soil and in older SOM-fractions than at present.

Our findings raise the question of when the savanna site first became dominated by C_4 grasses. The $\delta^{13}C$ signatures in the savanna's SOM fractions show that the DF had an unambiguous C_4 signature (−13.6‰; Fig. 5) but the more recalcitrant fractions did not. The ^{14}C-based mean age of this dense fraction was 1200 years. This implies that the savanna site, only 100 m from the current forest edge, has had a C_4-dominated vegetation at least since then. We assume that this is a rather conservative estimate, for two reasons. First, the radiocarbon-based mean ages of SOM result from a mixture of younger and older compounds. As the $\delta^{13}C$ signature of the DF was purely C_4-dominated, most of the older components of the fraction must have originated from savanna vegetation as well. Consequently, C_4 plants have been dominant for at least several centuries longer than the DF's mean age of 1200 years would suggest. Second, the negative $\delta^{13}C$ signal in SOM of the subsoils and old fractions (huminDF and recalcitrantDF; Fig. 5) of the savanna and ecotone could be due to the contribution of carbon-containing bedrock carrying a C_3 signature (e.g. Wilding *et al.*, 1996; Leavitt *et al.*, 2007). Although the lithological carbon content was small (0.13% carbon in the stone sample of the profile EE2), it could contribute up to 40% to the low soil carbon contents at greater depths of these relatively shallow savanna and ecotone soils due to the high density of the bedrock. As the stone contained a C_3 signature (−23‰), it might have shifted the $\delta^{13}C$ values towards an apparent higher contribution of C_3 plants (cf. Fig. 4 for the tendency of the savanna subsoils towards the background $\delta^{13}C$ value of the stone material). Consequently the question of whether the slightly depleted $\delta^{13}C$ value of the savanna's almost 2000-year-old huminDF really indicates the influence of former forest or whether the current savanna was

covered by C_4 grasses for much longer than the suggested 1200 years remains unanswered (Fig. 5).

A formerly greater contribution of C_3 trees to SOM in the currently open savanna would be supported by palynological studies suggesting that previously more extended tree formations in East Africa (as also in Western Equatorial Africa) were gradually replaced by grass and savanna vegetation (Kendall, 1969; Vincens *et al.*, 1999). This was probably due to the increasingly drier conditions about 4000 yr BP in this region (Gasse, 2000; Marchant & Hooghiemstra, 2004), which would agree with our data's timeline for the development of a C_4-dominated savanna in the past (Fig. 5, SE1).

Recent forest advances

Our stable isotope data of the fractionated SOM suggest that the proportion of trees at the ecotone between forest and savanna has recently increased. The light fractions (soilLF, oLF) of the ecotone profiles towards the savanna (e.g. EE2, EW3) show a strong isotopic depletion (up to 8‰) compared with the bulk soil and the dense fraction (Fig. 5). Consequently, the young SOM fractions, which reflect the input from the modern C_3-dominated vegetation system, are not in equilibrium with the long-term soil organic carbon pool at these two sites. We interpret this discrepancy as an indication that the trees have slowly gained more influence in the ecotone zone, and that the forest has thus been expanding into the savanna. These isotope-based findings of a slowly spreading Kagoma Forest are supported by three observations.

1. The presence of large abandoned *Macrotermes* mounds within the forest (about 50–150 m from the sharp forest edge). These termites live in savannas and woodlands and they are never found in closed-canopy forests (Harris, 1971), and thus, the termite mounds are relicts of a past open savanna environment.

2. The presence of typical savanna trees, like *Combretum molle*, which have become completely enclosed by the expanding forest. Such trees could only have been originally established within savannas, but they are now located as far as 20 m inside the forest.

3. Pollen profiles also provide evidence of the recent forest advances, but on a larger spatial scale. In the Tsavo National Park, east of Kilimanjaro, but also in Atlantic Equatorial Africa, the contribution of trees has increased during recent millennia (Vincens *et al.*, 1999; Gillson, 2004).

The isotopic signatures of bulk soil profiles sampled at the transition between the forest and savanna showed that the forest edge is remarkably sharp (Fig. 4 and Appendix S2). While the bulk SOM of the soil profile 5 m into the savanna showed almost the same $\delta^{13}C$ signature as the savanna soils 100 m from the forest, SOM in the profiles 5 m into the forest was clearly dominated by the forest vegetation throughout all depths. Consequently, the changes in the forested areas must be occurring very slowly. We could roughly assess the forest migration rate: the $\delta^{13}C$ signatures in the DF and the huminDF with a mean calibrated ^{14}C age of *c.* 1200 and *c.* 1850 years, respectively, have only changed significantly at the forest edge on a stretch that is less than 150 m long (Fig. 7). This suggests that the forest boundary has advanced by less than 15 m century^{-1} during at least the last millennium. Using a similar combined ^{13}C–^{14}C approach, Schwartz *et al.* (1996) estimated for the more humid Congolese Mayombe area that the mean rate of forest encroachment into enclosed savanna patches is 20 to 50 m century^{-1}. However, Schwartz *et al.* (1996) measured isotopes in bulk soils without removing the 'modern' light fraction, which results in younger ^{14}C ages, and thus apparently faster migration rates than in our study. Our estimate of a remarkably slow expansion of the Kagoma Forest is in agreement with the analyses of areal photographs in north-eastern Ivory Coast, where Goetze *et al.* (2006) observed that 95% of 650 forest islands in the savanna had remained unchanged during the last 50 years.

There appear to be several reasons for the very slow forest migration rate and high spatio-temporal stability of the forest–savanna boundary in the past. These include the frequent, but relatively cool savanna fires; the strong competition between grass vegetation and establishing tree seedlings; the browsing of young woody plants by cattle and game and the direct browsing impact on the forest edge by the elephants (*Loxodonta africana*) and black rhinoceros (*Diceros bicornis*) which were, until recently, widespread (Bloesch, 2002; Favier *et al.*, 2004; Hoffmann *et al.*, 2004). Moreover, edaphic changes may also contribute to the stable distribution of forest and savanna. Soils in the Kagoma Forest have a clayey loam texture and are deeply developed, while soils in the

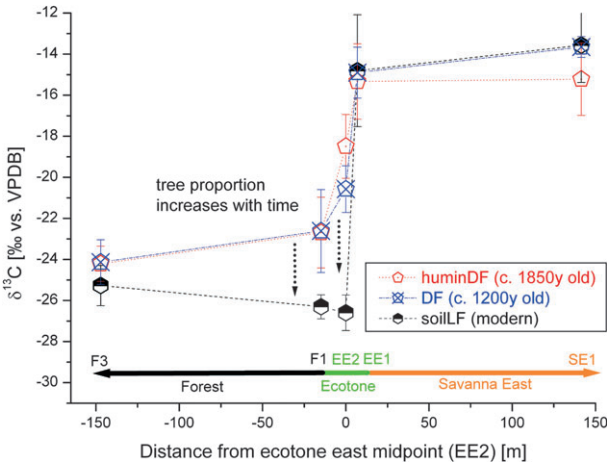

Figure 7 The change of $\delta^{13}C$ values across the eastern forest edge in the light fraction (soilLF) being radiocarbon-dated as 'modern' and the dense (DF) and humin dense (huminDF) fractions having a mean calibrated ^{14}C age of 1200 years and 1850 years, respectively. In the sharp ecotone, more negative $\delta^{13}C$ values in the LF than in the DF indicate that forest trees have slowly gained influence over the C_4 grasses in this section during at least the last millennium. Isotope results are reported in conventional δ notation as per mil (‰) relative to the carbon isotopic ratio of the VPDB standard (Fry, 2006). The study area is located in north-western Tanzania.

savanna exhibit a sandy loam texture and are shallower with a higher stone content. As the forest and savanna soils have evolved from the same parent material and because there is no apparent change in topography at the forest–savanna boundary (Fig. 1), we assume that the persistent tree cover over millennia has led to the stronger soil development in the forest. This vegetation-induced pedogenesis in turn provides more favourable conditions for tree growth inside the forest and in close proximity to it (i.e. at the forest edge) and could explain part of the very slow but nevertheless observable forest expansion with time. By contrast, the shallow savanna soils limit soil water storage and tree root penetration, and hence slow down the establishment of forest trees on former savanna ground. Similar reciprocal long-term effects between trees and their soil in an East African savanna environment were reported by Belsky & Amundson (1992).

The above considerations about a rather stable forest–savanna mosaic apply for the past only, because we think that the recently increasing human impact in the Kagera region is altering the controls on the forest–savanna boundary. Populations of elephants and black rhinoceros as the main browsers at the forest edge have strongly declined during recent decades (Bloesch, 2002). Furthermore, fires have become less frequent and changed from late to less intense early dry-season fires, which possibly promotes the expansion of the forest (Prior *et al.*, 2010). An increasing demand for fire wood, however, might lead to an increase in logging, which could easily outweigh all the other vegetation boundary processes in the near future.

CONCLUSIONS

1. Using soil fractionation according to soil density and chemical stability, it was possible to separate SOM fractions of distinct ages, allowing the reconstruction of past changes in the forest–savanna mosaic on a local scale.

2. We found no evidence for former C_4 vegetation at the inner forest sites, not even in the oldest SOM fractions with a mean calibrated ^{14}C age of 5500 years. Thus, the dry evergreen forests have existed since at least the mid-Holocene.

3. Savanna sites generally showed a typical C_4 isotopic signature in SOM, but their subsoils and more recalcitrant SOM fractions also carried the isotopic signature of C_3 plants. The radiocarbon data for the soil fractions suggest that the pure C_4 input had a mean calibrated ^{14}C age of approximately 1200 years. The actual length of time since the C_4 plants became dominant is probably longer because the dated fraction also contained older C_4-derived organic compounds. Furthermore, lithological carbon, which is unrelated to vegetation change, may have contributed to an apparent C_3 signature in the oldest fractions.

4. At the forest edge, the older C_4 grass signature of SOM at these sites has steadily been replaced by the more negative $\delta^{13}C$ fingerprint of the forest trees during at least the last millennium. However, as these changes occur across a sharp transition of only 10 m, the forest expansion must be very slow, and is very likely less than 15 m century^{-1}.

In summary, our results indicate that the savanna–forest mosaic in north-western-Tanzania has been rather stable since at least the mid-Holocene, and that the forest formations have been expanding at a very slow rate.

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REFERENCES

Accoe, F., Boeckx, P., Van Cleemput, O. & Hofman, G. (2003) Relationship between soil organic C degradability and the evolution of the $\delta^{13}C$ signature in profiles under permanent grassland. *Rapid Communications in Mass Spectrometry*, **17,** 2591–2596.

Asner, G.P., Levick, S.R., Kennedy-Bowdoin, T., Knapp, D.E., Emerson, R., Jacobson, J., Colgan, M.S. & Martin, M.E. (2009) Large-scale impacts of herbivores on the structural diversity of African savannas. *Proceedings of the National Academy of Sciences USA*, **106,** 4947–4952.

Balesdent, J. & Mariotti, A. (1996) Measurement of soil organic matter turnover using ^{13}C natural abundance. *Mass spectrometry of soils* (ed. by T.W. Boutton and S. Yamasaki), pp. 83–112. Marcel-Dekker, New York.

Belsky, A.J. & Amundson, R.G. (1992) Effects of trees on understorey vegetation and soils at forest–savanna boundaries. *Nature and dynamics of forest–savanna boundaries* (ed. by P.A. Furley, J. Proctor and J.A. Ratter), pp. 353–363. Chapman and Hall, New York.

Bloesch, U. (2002) *The dynamics of thicket clumps in the Kagera savanna landscape, East Africa*. PhD Thesis, Swiss Federal Institute of Technology, Zurich.

Bloesch, U. (2008) Thicket clumps: a characteristic feature of the Kagera savanna landscape, East Africa. *Journal of Vegetation Science*, **19,** 31–44.

Boutton, T.W., Archer, S.R., Midwood, A.J., Zitzer, S.F. & Bol, R. (1998) $\delta^{13}C$ values of soil organic carbon and their use in documenting change in a subtropical savanna ecosystem. *Geoderma*, **82,** 5–41.

Bowman, D.M.J.S., Boggs, G.S. & Prior, L.D. (2008) Fire maintains an *Acacia aneura* shrubland – *Triodia* grassland mosaic in central Australia. *Journal of Arid Environments*, **72,** 34–47.

Bronk Ramsey, C. (2001) Development of the radiocarbon program OxCal. *Radiocarbon*, **43,** 355–363.

Christensen, B.T. (1992) Physical fractionation of soil and organic matter in primary particle size and density separates. *Advances in Soil Science*, **20,** 1–90.

Durigan, G. & Ratter, J.A. (2006) Successional changes in cerrado and cerrado/forest ecotonal vegetation in western Sao Paulo State, Brazil, 1962–2000. *Edinburgh Journal of Botany*, **63,** 119–130.

Elenga, H., Peyron, O., Bonnefille, R. *et al.* (2000) Pollen-based biome reconstruction for southern Europe and Africa 18,000 yr BP. *Journal of Biogeography*, **27,** 621–634.

FAO (2006) *World reference base for soil resources 2006*. Food and Agriculture Organization, Rome.

Favier, C., Chave, J., Fabing, A., Schwartz, D. & Dubois, M.A. (2004) Modelling forest–savanna mosaic dynamics in man-influenced environments: effects of fire, climate and soil heterogeneity. *Ecological Modelling*, **171,** 85–102.

Favilli, F., Egli, M., Cherubini, P., Sartori, G., Haeberli, W. & Delbos, E. (2008) Comparison of different methods of obtaining a resilient organic matter fraction in Alpine soils. *Geoderma*, **145,** 355–369.

Fry, B. (2006) *Stable isotope ecology*. Springer, New York.

Gasse, F. (2000) Hydrological changes in the African tropics since the Last Glacial Maximum. *Quaternary Science Reviews*, **19,** 189–211.

Gillson, L. (2004) Evidence of hierarchical patch dynamics in an East African savanna? *Landscape Ecology*, **19,** 883–894.

Goetze, D., Horsch, B. & Porembski, S. (2006) Dynamics of forest–savanna mosaics in north-eastern Ivory Coast from 1954 to 2002. *Journal of Biogeography*, **33,** 653–664.

Harris, W.V. (1971) *Termites: their recognition and control*. Longman, London.

Higgins, S.I., Shackleton, C.M. & Robinson, E.R. (1999) Changes in woody community structure and composition under contrasting landuse systems in a semi-arid savanna, South Africa. *Journal of Biogeography*, **26,** 619–627.

Hoffmann, W.A., Orthen, B. & Franco, A.C. (2004) Constraints to seedling success of savanna and forest trees across the savanna–forest boundary. *Oecologia*, **140,** 252–260.

Jolly, D., Prentice, I.C., Bonnefille, R. *et al.* (1998) Biome reconstruction from pollen and plant macrofossil data for Africa and the Arabian peninsula at 0 and 6000 years. *Journal of Biogeography*, **25,** 1007–1027.

Kaiser, K. & Guggenberger, G. (2007) Distribution of hydrous aluminium and iron over density fractions depends on organic matter load and ultrasonic dispersion. *Geoderma*, **140,** 140–146.

Kendall, R.L. (1969) An ecological history of the Lake Victoria Basin. *Ecological Monographs*, **39,** 121–176.

Kgope, B.S., Bond, W.J. & Midgley, G.F. (2010) Growth responses of African savanna trees implicate atmospheric [CO_2] as a driver of past and current changes in savanna tree cover. *Austral Ecology*, **35,** 451–463.

Krull, E.G. & Bray, S.S. (2005) Assessment of vegetation change and landscape variability by using stable carbon isotopes of soil organic matter. *Australian Journal of Botany*, **53,** 651–661.

Krull, E.S., Skjemstad, J.O., Burrows, W.H., Bray, S.G., Wynn, J.G., Bol, R., Spouncer, L. & Harms, B. (2005) Recent vegetation changes in central Queensland, Australia: evidence from $\delta^{13}C$ and ^{14}C analyses of soil organic matter. *Geoderma*, **126,** 241–259.

Leavitt, S.W., Follet, R.F., Kimble, J.M. & Pruessner, E.G. (2007) Radiocarbon and δ 13C depth profiles of soil organic carbon in the U.S. Great Plains: a possible spatial record of paleoenvironment and paleovegetation. *Quaternary International*, **162–163,** 21–34.

Marchant, R. & Hooghiemstra, H. (2004) Rapid environmental change in African and South American tropics around 4000 years before present: a review. *Earth-Science Reviews*, **66,** 217–260.

Mikutta, R., Kleber, M., Kaiser, K. & Jahn, R. (2005) Review: Organic matter removal from soils using hydrogen peroxide, sodium hypochlorite, and disodium peroxodisulfate. *Soil Science Society of America Journal*, **69,** 120–135.

Mistry, J. (2000) *World savannas*. Prentice Hall, London.

Murage, E.W., Voroney, P. & Beyaert, R.P. (2007) Turnover of carbon in the free light fraction with and without charcoal as determined using the ^{13}C natural abundance method. *Geoderma*, **138,** 133–143.

Prior, L.D., Williams, R.J. & Bowman, J.S. (2010) Experimental evidence that fire causes a tree recruitment bottleneck in an Australian tropical savanna. *Journal of Tropical Ecology*, **26,** 595–603.

Pullan, R.A. (1979) Termite hills in Africa: their characteristics and evolution. *Catena*, **6,** 267–291.

Ratter, J.A. (1992) Transitions between cerrado and forest vegetation in Brazil. *Nature and dynamics of forest–savanna boundaries* (ed. by P.A. Furley, J. Proctor and J.A. Ratter), pp. 417–429. Chapman and Hall, London.

Rethemeyer, J., Kramer, C., Gleixner, G., John, B., Yamashita, T., Flessa, H., Andersen, N., Nadeau, M.-J. & Grootes, P.M. (2005) Transformation of organic matter in agricultural soils: radiocarbon concentration versus soil depth. *Geoderma*, **128,** 94–105.

Rodgers, W.A., Ludanga, R.I. & DeSuzo, H.P. (1977) Biharamulo, Burigi and Rubondo Island Game Reserves. *Notes and Records*, **81,** 99–123.

Roques, K.G., O'Connor, T.G. & Watkinson, A.R. (2001) Dynamics of shrub encroachment in an African savanna: relative influences of fire, herbivory, rainfall and density dependence. *Journal of Applied Ecology*, **38,** 268–280.

Roscoe, R., Buurman, P. & Velthorst, E.J. (2000) Disruption of soil aggregates by varied amounts of ultrasonic energy in fractionation of organic matter of a clay Latosol: carbon, nitrogen and $\delta^{13}C$ distribution in particle-size fractions. *European Journal of Soil Science*, **51,** 445–454.

Schlüter, T. (2006) *Geological atlas of Africa*. Springer, Berlin.

Schmidt, M.W.I., Rumpel, C. & Kögel-Knabner, I. (1999) Evaluation of an ultrasonic dispersion procedure to isolate primary organomineral complexes from soil. *European Journal of Soil Science*, **50,** 87–94.

Scholes, R.J. & Archer, S.R. (1997) Tree-grass interactions in savannas. *Annual Review of Ecology and Systematics*, **28,** 517–544.

Schwartz, D., de Foresta, H., Mariotti, A., Balesdent, J., Massimba, J.P. & Girardin, C. (1996) Present dynamics of the savanna-boundary in the Congolese Mayombe: a pedological, botanical and isotopic (^{13}C and ^{14}C) study. *Oecologia*, **106,** 516–524.

Stuiver, M. & Pollach, H.A. (1977) Discussion: reporting of ^{14}C data. *Radiocarbon*, **19,** 355–363.

Troupin, G. (1966) *Etude phytocénologique du Parc National de l'Akagera et du Rwanda Oriental: recherche d'une méthode d'analyse appropriée à la végétation d'Afrique Intertropicale*. Publication No. 2, Institut National de Recherche Scientifique, Butare.

Vincens, A., Schwartz, D., Elenga, H., Reynaud-Farrera, I., Alexandre, A., Bertaux, J., Mariotti, A., Martin, L., Meunier, J.-D., Nguetsop, F., Servant, M., Servant-Vildary, S. & Wirrmann, D. (1999) Forest response to climate changes in Atlantic Equatorial Africa during the last 4000 years BP and inheritance on the modern landscapes. *Journal of Biogeography*, **26,** 879–885.

Wilding, L.P., Yao, L., Nordt, L.C., Drees, L.R., Archer, S. & Boutton, T.W. (1996) Soil, geomorphology, and vegetation relationships. *La Copita Research Area consolidated progress report*. Texas Agricultural Experiment Station, College Station, TX.

Wynn, J.G., Bird, M.I. & Wong, V.N.L. (2005) Rayleigh distillation and the depth profile of $^{13}C/^{12}C$ ratios of soil organic carbon from soils of disparate texture in Iron Range National Park, Far North Queensland, Australia. *Geochimica et Cosmochimica Acta*, **69,** 1961–1973.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Appendix S1 (a) Typical plant species in the Kagoma landscape and their cover-abundance and (b) photographs taken from the savanna towards the forest.

Appendix S2 Sharp change in $\delta^{13}C$ values in the narrow ecotone.

Appendix S3 ^{14}C measurements table.

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