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Long-term impacts of anthropogenic perturbations on dynamics and speciation of organic carbon in tropical forest and subtropical grassland ecosystems

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Abstract

Anthropogenic perturbations have profoundly modified the Earth's biogeochemical cycles, the most prominent of these changes being manifested by global carbon (C) cycling. We investigated long-term effects of human-induced land-use and land-cover changes from native tropical forest (Kenya) and subtropical grassland (South Africa) ecosystems to agriculture on the dynamics and structural composition of soil organic C (SOC) using elemental analysis and integrated ^{13}C nuclear magnetic resonance (NMR), near-edge X-ray absorption fine structure (NEXAFS) and synchrotron-based Fourier transform infrared-attenuated total reflectance (Sr-FTIR-ATR) spectroscopy. Anthropogenic interventions led to the depletion of 76%, 86% and 67% of the total SOC; and 77%, 85% and 66% of the N concentrations from the surface soils of Nandi, Kakamega and the South African sites, respectively, over a period of up to 100 years. Significant proportions of the total SOC (46–73%) and N (37–73%) losses occurred during the first 4 years of conversion indicating that these forest- and grassland-derived soils contain large amounts of labile soil organic matter (SOM), potentially vulnerable to degradation upon human-induced land-use and land-cover changes. Anthropogenic perturbations altered not only the C sink capacity of these soils, but also the functional group composition and dynamics of SOC with time, rendering structural composition of the resultant organic matter in the agricultural soils to be considerably different from the SOM under natural forest and grassland ecosystems. These molecular level compositional changes were manifested: (i) by the continued degradation of O-alkyl and acetal-C structures found in carbohydrate and holocellulose biomolecules, some labile aliphatic-C functionalities, (ii) by side-chain oxidation of phenylpropane units of lignin and (iii) by the continued aromatization and aliphatization of the humic fractions possibly through selective accumulation of recalcitrant H and C substituted aryl-C and aliphatic-C components such as (poly)-methylene units, respectively. These changes appeared as early as the fourth year after transition, and their intensity increased with duration of cultivation until a new quasi-equilibrium of SOC was approached at about 20 years after conversion. However, subtle but persistent changes in molecular structures of the resultant SOM continued long after (up to 100 years) a steady state for SOC was approached. These molecular level changes in the inherent structural composition of SOC may exert considerable influence on biogeochemical cycling of C and bioavailability of essential nutrients present in association with SOM, and may significantly affect the sustainability of agriculture as well as potentials of the soils to sequester C in these tropical and subtropical highland agroecosystems.

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Introduction

Human activities have fundamentally altered many of the Earth's biogeochemical cycles, the most prominent of these changes being manifested by modification of the global C cycle. Over the past two centuries alone, anthropogenic perturbations have led to a 31% increase in atmospheric CO_2 concentration from a preindustrial level of about 280 ppmv in 1800 to 368 ppmv in 2000 (IPCC, 2001). Most of this anthropogenic CO_2 enrichment came from burning of fossil fuel (270 Pg C), followed by emissions from activities related to land-use and land-cover changes (136 Pg C; Houghton, 1999; Lal, 2003). Of the emissions from land-use and land-cover changes, about 78 Pg C originated from depletion of soil organic matter (SOM; Lal, 2003).

Soil contains the largest pool of terrestrial organic C (1500 Pg C) in the biosphere storing more C than is contained in the biotic (560 Pg C) and atmospheric (760 Pg C) pools combined (Batjes, 1996; Schlesinger, 1997). In undisturbed terrestrial ecosystems, each soil has a C carrying capacity [i.e. equilibrium soil organic C (SOC) content] depending on the climate, vegetation, topography, parent material and time. In such ecosystems, the biogeochemical cycling of C is essentially in balance with minimal short-term losses or gains. The steady state attained in such ecosystems and thereby the amount, structural composition and stability of SOC pools, however, can be dramatically influenced by anthropogenic land-use and land-cover changes that reduce organic matter inputs and affect rates and processes underlying the equilibrium state until a new steady state is eventually established in the new ecosystem (Guo & Gifford, 2002; Solomon *et al*., 2005a). The principal types of these land-use and land-cover changes involve clearing of natural forest and grassland ecosystems for agricultural purposes. Current estimates indicate that these practices have led to a global increase in the total area of cultivated land by more than 425% since 1850, with the most rapid changes occurring in tropical and subtropical regions especially after the 1950s (Houghton, 1999). Given that one-third of the global SOC pool is in the tropics (Eswaran *et al*., 1993), these anthropogenic disturbances feedback on the global C cycle by increasing CO_2 flux from the soil to the atmosphere, and are expected to have consequences on the Earth's climate and biogeochemical cycling of other elements. SOM is also an important determinant of soil fertility in tropical and subtropical agroecosystems and its loss will have profound implications on soil productivity and sustainability of agriculture in these ecosystems. Hence, there is an urgent need to improve our understanding of the effects of these anthropogenic perturbations on the reactivity, fate and chemical speciation of SOC, as well as the mechanisms and processes that control its stability and determine the potentials of soils to sequester C in tropical and subtropical ecosystems. As the dynamics of SOC are included in global C cycle scenarios using different, but generally arbitrarily defined kinetic pools (Gleixner *et al*., 2002), detailed studies about structural chemistry and kinetic pools of SOM following net C losses due to land-use changes in different ecosystems across the globe may help to register changes in soil quality and land degradation, as well as to improve global C cycling and ecosystem models.

The impacts of land-use and land-cover changes on SOC in the tropics and subtropics have been the focus of substantial research in the past. Various studies indicated that land conversion from natural forest or grassland to agriculture is pervasive and leads to a reduction of up to 58% of SOC pool in the tropics (Nye & Greenland, 1964; Dalal & Mayer, 1986a; Davidson & Ackerman, 1993). Although the processes of SOC sequestration and destabilization may be controlled at the molecular level, most of these studies focused only on the absolute amounts of SOC without much recourse to the subtle changes that occur in the structural composition of SOM at the molecular level, as well as the long-term ecological significance of these changes in the tropics and subtropics (Martens *et al*., 2003; Piccolo *et al*., 2004). Moreover, among the available studies, the impact of land-use changes on SOM composition were often not clear; some studies suggested anthropogenic management imparts little impact, while others have indicated significant changes in the structural composition of SOM (Skjemstad & Dalal, 1987; Zech *et al*., 1997; Solomon *et al*., 2000, 2002; Lobe *et al*., 2002; Pérez *et al*., 2004). Gleixner *et al*. (2002) and Kögel-Knabner (2000) stated that most of the discrepancy is related to the complex nature of SOM and to analytical limitations of the methods employed to effectively characterize SOM and follow its dynamics in soils.

SOM is a heterogeneous mixture composed of organic molecules representing both compounds released from living plant and microbial cells (e.g. extracellular en-

zymes, surface-active proteins, chelating compounds, etc.) to complex plant, animal and microbial residues ranging in size and complexity from simple monomers or organic acids to mixtures of complex biopolymers that differ in stability. Such variations along the decomposition and size continuum create significant analytical problems and thus have made studies on SOM composition and its implications for the global biogeochemical cycling of C very challenging. These challenges have led to recent advances in analytical techniques that advanced our understanding of the processes underlying its transformations forcing us to re-examine some of the long-held theories about SOM (Hatcher *et al*., 2001). Despite these advances, Kögel-Knabner (2000, 2002) emphasized that SOM characterization could further benefit from the progress made in nondestructive microscopic and microscale X-ray spectroscopy techniques to gain new insights about the reactivity, composition, microheterogenity and physical location of organic materials in soils. Recent investigations using synchrotron-based scanning transmission X-ray microscopy (STXM), C (1s) near-edge X-ray absorption fine structure (NEXAFS) and synchrotron-based Fourier transform infrared-attenuated total reflectance (Sr-FTIR-ATR) spectroscopy have indicated that these techniques are powerful, noninvasive techniques methods, which can be used to identify and fingerprint the complex structural characteristics of SOC, as well as to investigate the impact of management on the composition and biogeochemical cycling of organic C at the molecular level in terrestrial ecosystems (Scheinost *et al*., 2001; Jokic *et al*., 2003; Schäfer *et al*., 2003; Lehmann *et al*., 2005; Solomon *et al*., 2005b).

Therefore, the objectives of this study were: (i) to identify and fingerprint the functional group composition of SOC using C K-edge NEXAFS and Sr-FTIR-ATR spectroscopic techniques, and (ii) to investigate the long-term impact of anthropogenic land-use and land-cover changes on the amount and molecular level speciation of SOM in up to 100 years old agricultural fields converted from native tropical forest and subtropical grassland ecosystems of Kenya and South Africa, respectively. The results from C (1s) NEXAFS and Sr-FTIR-ATR spectroscopy were evaluated against the results obtained from ^{13}C nuclear magnetic resonance (NMR) spectroscopy, which is a more established SOC characterization technique.

Materials and methods

Site description

The study was conducted using soil samples collected from the Kakamega (00°14′19″N, 34°57′13″E) and South Nandi (00°04′30″N, 34°58′34″E) forests of western Kenya and from the grassland ecosystems near Harrismith (29°7′60″E, 28°16′60″S), Kroonstad (27°13′60″E, 27°38′60″S) and Tweespruit (26°10′0″E, 29°46′0″S) in South Africa. The Kakamega forest is the eastern-most remnant of Guineo-Congolian rainforest, which in the past millennium stretched across the entire expanse of West and central Africa to East African highlands. It is one of the last remnants of virgin tropical rainforests currently existing in this intensely cultivated region. The Kakamega forest was a contiguous forest until 1895; since then, the forested area has been constantly decreasing through deforestation to a number of peripheral fragments among which the Nandi highland forests are the largest ones. The altitude of the Kakamega and Nandi sites ranges from 1700 to 1800 m above sea level (a.s.l.). Mean annual temperature (MAT) is about 19 °C with mean annual precipitation (MAP) of 2000 mm. The soils of Kakamega forest are well-drained, deep red to yellowish red, friable sandy clay to sandy loam texture. They are developed from undifferentiated Basement System rocks and are classified as Ferralo-Chromic Acrisols (FAO-UNESCO, 1997). The southern Nandi forest is composed of well-drained, extremely deep and dark to reddish brown soils with friable clay and thick humic top layer principally developed on biotite-gneiss parent material. They are classified as Humic Nitosols (FAO-UNESCO, 1997). The natural vegetation of these two sites is composed of tropical rainforest of Guineo-Congolian species, including *Aningeria altissima* (A. Chev.), *Milicia excelsa* (Welw., C. C. Berg), *Antiaris toxicaria* (Lesch) and *Chrysophyllum albidum* (G. Don). There are also species of montane forest including *Olea capensis* (L.) and *Croton megalocarpus* (Hutchinson). The agricultural fields at the Kakamega and Nandi sites were plowed to 10–12 cm depth, and maize (*Zea mays* L.) was grown as the main crop without fertilizer inputs with an occasional inclusion of finger millet (*Eleusine coracana* Gaertn.) or sorghum [*Sorghum bicolor* (L., Moench)]. At both sites, crop residues are collected and used as animal feed.

The altitude of the South African sites ranges from 1350 to 1800 m a.s.l. The three sites are located in the summer rainfall region with MAP ranging from 516 to 625 mm and MAT ranging from 14 to 17 °C. They belong to the Highveld grassland biome, which is dominated by *Cymbopogon plurinodis* (Stapf ex Burtt-Davy), *Themeda triandra* (Forssk.), *Elionurus muticus* (Spreng., Kuntze) and *Eragrostis curvula* (Schrad., Nees) at Harrismith; *E. lehmanniana* (Nees), *E. obtusa* (Munro ex Ficalho and Hiern), *Panicum coloratum* (L)., *Stipagrostis uniplumis* (Licht., De Winter) and *Pentzia globosa* (Less.) at Kroonstad, and *T. triandra* (Forssk.) at Tweespruit. The soils have medium to coarse texture and are classified as

Dystric to Eutric Plinthosols (FAO-UNESCO, 1997). The agricultural fields were plowed to a depth of 20–30 cm and wheat (*Triticum aestivum* L.) and maize (*Z. mays* L.) were grown in rotation with inorganic fertilizers.

Sampling

In western Kenya, we selected sites from the natural forests and from fields cultivated for 2, 4, 20, 30, 50, 80, 100 years at the Nandi and for 2, 4, 18, 45, 73 and 103 years at the Kakamega sites. Similarly, in South Africa, we sampled soils from arable land under cultivation for about 3, 8, 10, 20, 30, 40, 60 and 90 years and from adjacent native grasslands sites in the three agroecosystems. At each field, we collected nine 200 cm^3 core subsamples from the upper 10 cm soil (upper 20 cm in the South African sites) in a radial sampling scheme, which were later combined to one composite sample. In some sites, we were able to locate more than one land-use transitions from the same age group, and we collected replicate samples from such fields. However, due to limitations in instrument availability and beam-time allocation, we pooled these replicate samples together (in the case of the South African sites, we mixed samples collected from the three sites that belong to the same age group together) and prepared one representative sample per site to represent each conversion time. The samples were then air dried and sieved (<2 mm) before chemical analysis.

Chemical analysis

Total C, N and H concentrations in the bulk soils and humic fractions were determined with a C/H/N/S-analyzer (Elementar Analysensysteme GmbH, Hanau, Germany). The concentration of O in the humic fractions was measured by PerkinElmer 2400 elemental analyzer (PerkinElmer Life and Analytical Sciences Inc., Wellesley, MA, USA). The pH-H_2O and pH-KCl were determined in a 1 : 2.5 soil : water (w/v) suspension. Selected soil physical and chemical characteristics of the sites are shown in Table 1.

Extraction of humic fractions

Humic fractions are complex, dark colored, heterogeneous mixtures of organic materials which comprise both humic substances and recognizable biomolecular fragments intimately associated even covalently bonded with humic substances (Sutton & Sposito, 2005). They were extracted from the soils three times with a mixture of 0.1 M NaOH and 0.4 M NaF (pH 12.4) at a soil to extraction solution ratio of 1 : 5 (w/v) under N_2 environment. The extraction procedure followed the outline of Schnitzer (1982), as modified by (Sumann *et al*., 1998). Replacement of 0.1 M $Na_4P_2O_7$ by 0.1 M NaOH–0.4 M NaF mixture does not affect the ^{13}C NMR spectra of humic fractions, rather it improves extraction yield compared with extraction using only 0.1 M $Na_4P_2O_7$ (Sumann *et al*., 1998). The F^- ion was introduced to dissolve silicate impurities and reduce the influence of paramagnetic metals that are major impediments to the use of solid-state ^{13}C NMR spectroscopy for the characterization of SOM in whole soils and humic fractions (Kim *et al*., 1990; Smernik & Oades, 2002). Combined extracts were filtered twice through a 0.2 μm membrane filter (Gelman Supor, Pall Gelman Laboratory, Ann Arbor, MI, USA) under pressure to remove fine clay, that may interfere with the NEXAFS and ^{13}C NMR measurements (Solomon *et al*., 2005b). The extracts were transferred into dialysis tubes (MWCO 12 000–14 000 Da; Spectrum Laboratories, Gardena, CA, USA) and dialyzed against distilled–deionized water to remove salts and finally lyophilized using a freeze dryer (Kinetics Thermal Systems, Stone Ridge, NY, USA). This procedure extracted 51–72% of the SOM from the bulk soils, and is within the ranges reported by Rice (2001) and Scheffer & Schachtschabel (2002).

Table 1 Selected climate and physical and chemical characteristics of the surface soils from Kenya and South Africa

Site	Land use	Longitude	Latitude	Altitude (m)	MAT (°C)	MAP (mm)	g kg^{-1} soil			BD (g cm^{-3})	pH		g kg^{-1} soil		
							Sand	Silt	Clay		H_2O	KCl	SOC	N	C/N
Kenya															
Nandi	Forest	00°04′N	34°58′E	1800	19.6	2000	570	180	240	0.65	6.5	6.0	95.1	9.5	10.1
Kakamega	Forest	00°14′N	34°57′E	1703	19.0	2080	400	210	380	0.76	5.9	5.3	118.7	10.8	11.0
South Africa															
Harrismith	Grassland	28°16′S	29°7′E	1753	13.8	625	750	100	150	1.28	5.2	4.5	20.6	1.60	12.9
Kroonstad	Grassland	27°38′S	27°13′E	1416	16.6	563	830	50	120	1.39	6.0	5.0	8.0	0.85	9.4
Tweespruit	Grassland	29°46′S	26°10′E	1379	16.0	516	770	100	130	1.30	5.9	5.0	11.7	1.12	10.5

MAT, mean annual temperature; MAP, mean annual precipitation; BD, bulk density; SOC, soil organic carbon.

^{13}C NMR spectroscopy

Solid-state ^{13}C cross polarization-magic angle spinning (CP-MAS) spectra from humic fractions extracted from the Kenyan soils were obtained at a frequency of 50.318 MHz on a Varian Unity 200 spectrometer (Varian Inc., Palo Alto, CA, USA) with a 4.7 T wide-bore Oxford superconducting magnet. About 200 mg of humic fraction samples were packed in 7 mm diameter zirconia rotors with Kel-F caps and spun at 5 kHz in Doty Scientific MAS probe (Doty Scientific Inc., Columbia, SC, USA). All spectra were attained with a contact time of 1 ms and recycle delay time of 500 ms to ensure complete relaxation between scans (recycle delay >7 T_1H). The spectra were plotted between −100 and 300 ppm using a Lorentzian line broadening of 50 kHz and other parameters as described by Skjemstad *et al*. (2001). Signal recovery for the samples in CP mode ranged from 48–72% (i.e. 67% in natural forest, 56% in 2 years cultivated, 56% in 20 years cultivated, 54% in 50 years cultivated and 55% in 100 years cultivated fields at Nandi, and 64% in natural forest, 72% in 2 years cultivated, 56% in 18 years cultivated, 48% in 45 years cultivated, 59% in 73 years cultivated and 53% in 103 years cultivated fields at Kakamega), indicating relatively low recovery of signals common to humic fractions. Owing to insufficient quantity of humic fractions required for solid-state ^{13}C CP-MAS NMR spectroscopy of the South African samples, we used previously recorded liquid-state ^{13}C NMR spectra from these samples in the current investigation. Liquid-state ^{13}C NMR spectra were recorded as described by Solomon *et al*. (2002) on a Bruker Avance DRX 500 NMR spectrometer (Bruker BioSpin GmbH, Rheinstetten, Germany). Chemical shifts were recorded in ppm relative to the resonance of an external TSP (3-trimethylsilyl propionic) acid standard and signal areas were recalculated to match the chemical shift regions used in the ^{13}C CP-MAS NMR results. The humic fractions used for ^{13}C NMR analysis were not pretreated with dilute hydrofluoric acid (HF) to minimize SOC loss and possible changes in amino acid and carbohydrate structures following pretreatment (Gonçalves *et al*., 2003), as well as to maintain sample uniformity used in all spectroscopy techniques.

Sr-FTIR spectroscopy

Sr-FTIR-ATR spectra were recorded on U10B beamline at the National Synchrotron Light Source (NSLS), Brookhaven National Laboratory. The beamline is equipped with a Spectra Tech Continuum IR microscope fitted with ×32 transmission/reflection and FTIR step-scan spectrophotometer (Nicolet Magna 860, Thermo Nicolet Corporation, WI, USA) with a KBr beam splitter and mercury–cadmium–telluride (MCT) detector with 500–7000 cm^{-1} frequency range and 1.0 cm^{-1} spectral resolution. Thin films of humic fraction samples were prepared as described in Solomon *et al*. (2005b) from the aqueous suspension obtained by dispersing them in millipore water using ultrasound bath. We transferred 10 μL droplets of the aqueous suspension on MirrIR glass slides (Kevley Techniologies, Chesterland, OH, USA) and the suspension was dried at 35 °C in a vacuum oven. FTIR-ATR spectra from the samples were recorded with a 10 μm × 10 μm aperture size from 4000 to 650 cm^{-1} with a resolution of 4 cm^{-1}. Each spectrum was composed of 256 scans coadded before Fourier transform processing. After subtracting the background of the IR glass, we used OMNIC version 6.1 for windows (Thermo Nicolete Corp.) on the reduced portions of the spectra (4000–800 cm^{-1}) to automatically correct the baseline, normalize, identify the peaks and calculate the signal intensities of the spectra.

NEXAFS

C (1s) NEXAFS were recorded at X-1A1 beamline of the NSLS using the STXM endstation. The essential components of the STMX used in the present experiment were a tunable undulator, which is inserted in the 2.8 GeV electron storage ring generating a high flux photons at 10^6 spatially coherent photons s^{-1} in the soft X-ray region, a spherical grating monochromator with maximum spectra resolving power of 5000 lines mm^{-1}, a 160 μm Fresnel zone plate with a normal spatial resolution of 45 nm and a proportional counter to detect the transmitted photons. The beamline slit width was set to 45 μm × 25 μm × 25 μm. The monochromator was calibrated using the absorption band of CO_2. Thin films were prepared from the aqueous suspension of humic fractions in similar manner as for Sr-FTIR-ATR. We transferred 3 μL droplets of the aqueous suspension to 100 nm thick Si_3N_4 windows (Silson Ltd., Northampton, UK) and dried the suspension at 35 °C. After high-resolution micrographs were taken by STXM to locate an area of uniform thickness, the illuminated spot on the samples were then increased to 10 μm by defocusing the zoneplate. Spectra from the samples (I) were collected from three different spots through the films and Si_3N_4 windows by moving the grating from 280 to 310 eV on a single spot with 120 ms dwell times in energy steps of 0.1 eV. Before each sample scan, background spectra (I_o) were collected in triplicates from the sample free region of the same Si_3N_4 windows and averaged. Two ionization thresholds (IP) were set for the NEXAFS spectra deconvolution by setting two arctangent functions (AT) at 290.5 eV for aromatic/

aliphatic C and 292.0 eV for hydroxylated aromatic C (taking into account the C and O composition of the samples) with full-width at half-maximum (FWHM) of 0.4 eV to generate a continuum of spectrum up to 294 eV. The FWHM of the Gaussian peaks (G) was set at 0.4 eV and six Gaussian functions representing the main 1s–π^* transitions at 284.4 (G1), 285.2 (G2), 286.6 (G3), 287.6 (G4), 288.5 (G5) and 289.3 (G6) eV were resolved. Furthermore, two σ^* transitions (290.2, ζ^1 and 291.5, σ^2) were simulated by simplified Gaussian shape function with FWHM of <1 and <2 eV, respectively, using WinXAS version 3.1 for windows (WinXAS Software, Hamburg, Germany). The spectra were pre-edge normalized using WinXAS to avoid spectral dependence on C content, therefore, spectral properties are indicative of changes in C chemistry. As fine structures in the C NEXAFS region above 290 eV transitions tend to be very broad and overlap with each other (Cody *et al*., 1998; Schäfer *et al*., 2003), only main 1s–π^* transitions were used for quantification and interpretation of the NEXAFS results in the present investigation.

Decay models and statistics

Based on the assumption that SOM reaches new equilibrium concentration following land-use changes, Dalal & Mayer (1986a) and Lobe *et al*. (2001) used exponential models to describe the response of organic matter in soils following cultivation. We employed similar approaches and used a monoexponential decay model to describe the dynamics of SOC and N concentrations following long-term land-use changes in the surface soils under investigation:

$$X_t = X_e + (X_0 - X_e)\exp(-kt), \quad (1)$$

where X_t is the concentration of SOC and N in the soils at cultivation time t, X_e is the total SOC and N concentration in the soils at equilibrium, X_0 is the initial concentration of SOC and N in the native forest or grassland soils ($t = 0$), and k is a rate constant (year^{-1}). Additionally, as different rates of SOM decomposition have frequently been attributed to biologically meaningful discrete pools of varying stability (Jenkinson & Rayner, 1977), we used a modified version of a bi-exponential model (Lobe *et al*., 2001) to describe the dynamics of the different SOC and N pools in the investigated soils:

$$X_t = X_1\exp(-k_1t) + X_2\exp(-k_2t), \quad (2)$$

where X_t is the concentration of total SOC and N at cultivation time t, X_1 is the concentration of SOC and N of the labile pool, X_2 is the concentration of SOC and N of the stable pool ($X_2 = X_0 - X_1$), k_1 is the rate constant of the labile pool (year^{-1}), and k_2 is the rate constant of the stable pool (year^{-1}). The data set for the SOC and N concentrations ($n = 8$ at Nandi, $n = 7$ at Kakamega and $n = 9$ at the South African sites) in our experiment were fairly small and would have resulted in overparameterization if used to calculate SOC and N concentrations at equilibrium (X_e) using extended biexponential model [$X_t = X_1\exp(-k_1t) + X_2\exp(-k_2t) + X_e$]. Therefore, we did not determine the equilibrium concentrations of total SOC and N following cultivation using the biexponential model. To reduce variability between SOM concentrations in the different agroecosystems, SOC and N losses were expressed as relative proportions to total SOC and N of the natural forest and grassland ecosystems (i.e. $X_0 = 100$). Correlation coefficients showing the relationship between the loss of total SOC and N as well as the relationship between the band areas of organic C functional groups derived from C (1s) NEXAFS, ^{13}C NMR and signal intensities of the Sr-FTIR-ATR spectroscopy of humic fractions were run by Pearson product moment correlation using Statistica 5.0 for Windows (1999).

Results and discussions

Organic C and N concentrations and long-term dynamics in bulk soils

The concentration of SOC in surface layers of the forest-derived soils of Kenya varied from 15.3 to 95.1 g kg^{-1} soil at Nandi and from 16.8 to 118.7 g kg^{-1} soil at Kakamega, while the concentration of total N ranged from 1.51 to 9.5 g kg^{-1} soil and from 1.5 to 10.8 g kg^{-1} soil at the two sites, respectively. These values compare positively with the ranges SOC and N concentrations reported for tropical forest-derived soils by Krull *et al*. (2002) and Nziguheba *et al*. (2005) from Kenya, Guggenberger *et al*. (1999) from Costa Rica, Möller *et al*. (2000) from Thailand, and by Solomon *et al*. (2002) and Lemenih & Itanna (2004) from Ethiopia. The concentrations of SOC and N in the grassland-derived soils of South Africa ranged from 5.3 to 16.3 g kg^{-1} soil and from 0.60 to 1.4 g kg^{-1} soil, respectively, and are considerably lower than the values from forest-derived soils of Kenya. However, they are in line with the ranges reported for variety of tropical and subtropical grassland-derived soils by Bechtold & Naiman (2006) from South Africa, Zingore *et al*. (2005) from Zimbabwe, Agbenin & Adeniyi (2005) from Nigeria, Solomon *et al*. (2000) and McDonagh *et al*. (2001) from Tanzania, and by Michelsen *et al*. (2005) from Ethiopia. The higher SOC and N concentrations in the subhumid tropical agroecosystems of Kenya compared with the subtropical grassland agoecosystems of South Africa could be attributed to differences in climate, the quantity and

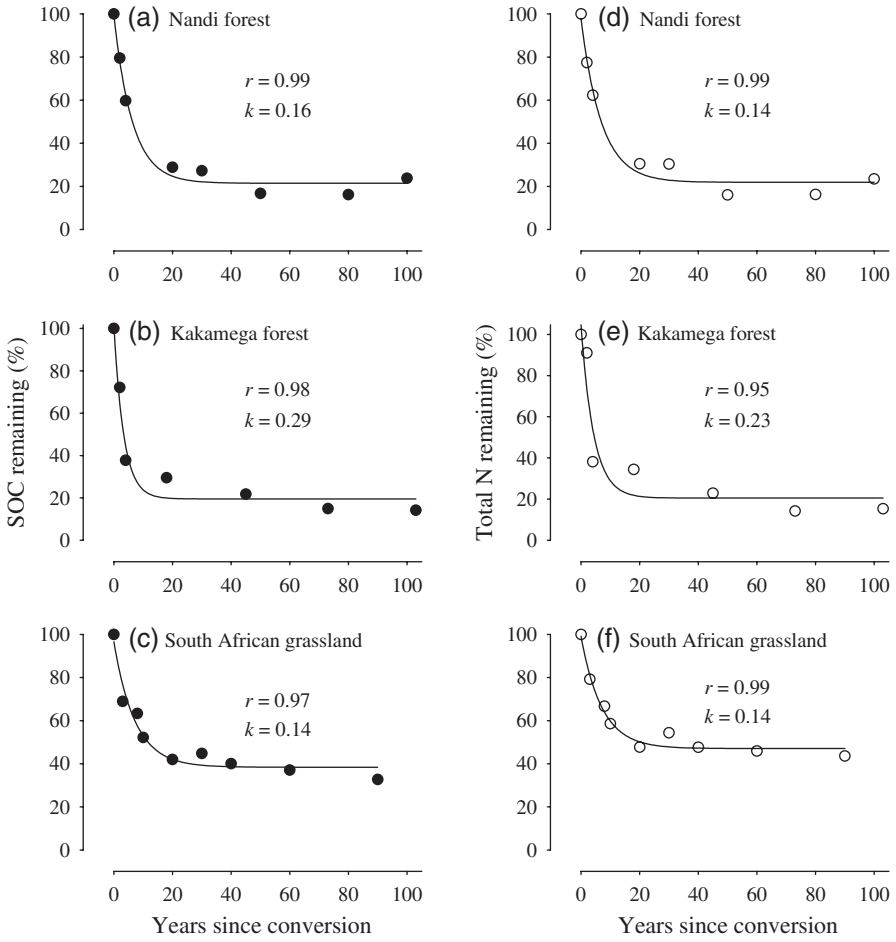

Fig. 1 Long-term impacts of human-induced land-use and land-cover changes on the concentrations of total organic carbon and N in the surface layers of soils from the tropical forest agroecosystems of Kenya and subtropical grassland agroecosystems of South Africa. The solid lines represent fit using monoexponential model [Eqn (1)].

quality of organic matter produced and incorporated annually in to the soil, and to variations in sampling depth and soil type.

The proportions of SOC and N that remained in the surface soils in relation to the duration of cultivation are shown in Fig. 1. According to this figure, the concentration of organic C in the forest-derived soils of Nandi and Kakamega, as well as in the grassland-derived soils of South Africa decreased exponentially to about 24%, 14% and 33% of the original amount following clearing of the natural vegetation and long-term cultivation, respectively. Similarly, the concentration of total N declined with increasing duration of cultivation to about 23%, 15% and 44% of the original concentration at Nandi, Kakamega and the South African sites, respectively, over a period of up to 100 years. Significant proportions of the total SOC (46–73%) and N (37–73%) losses occurred during the first 3–4 years of conversion indicating that the surface layers of these forest- and grassland-derived soils contain large amount of labile SOM, which is potentially very vulnerable to degradation upon human-induced land-use and land-cover changes. Our results show that SOC and N concentrations in the arable soils approached steady-state equilibrium (t_e) after about 37 and 38 years at Nandi, after about 21 and 29 years at Kakamega and after about 34 and 30 years at the South African sites, respectively. The rate and magnitude of changes in SOC and N observed especially at the early stages of conversion in these tropical and subtropical highland agroecosystems are much larger than the values reported by Bowman *et al*. (1990) and Davidson & Ackerman (1993) for temperate soils and by Nye & Greenland (1964), Solomon *et al*. (2000) and Dieckow *et al*. (2005) for a range of tropical soils.

As more detailed information about the dynamics of SOC and N pools can be obtained using biexponential model, we calculated the time required to reach the point of kinetic change (t_{kc}) at which losses from the

stable pool started to control the dissipation kinetics using Eqn (2). The results indicated that for the total SOC concentration, this point was reached after 28, 13 and 17 years of cultivation at Nandi, Kakamega and the South African sites, respectively; whereas for total N, it took 24 years at Nandi, 15 years at Kakamega and 22 years at the South African sites. Hence, we suggest that the capacity of these natural forest- and native grassland-derived soils to supply easily mineralizable SOM pools and associated essential plant nutrients declined considerably on average after about 20 years of continuous cropping. We attribute the rapid depletion of SOC and N at the early stage of transition to enhanced biological mineralization SOM as a result of improved soil aeration and exposure of physically protected organic materials in aggregates to soil microorganism due to physical disruption. Moreover, reduced input of plant residues into the surface soils, as well as accelerated loss of soil by erosion, and hence, C and N from the surface soils due to land-use and land-cover changes (Zingore *et al*., 2005) may have also contributed to the observed depletion of SOC and N from the agriculturally managed fields. Our results also indicate that the forest-derived soils of Kenya lost larger proportions of SOC and N compared with the grassland-derived soils of South Africa. This might be ascribed to higher initial SOM content accompanied by relatively higher MAT and MAP of the western Kenyan sites, which create conducive environment for decomposers and increases vulnerability of SOM to oxidative mineralization and other losses associated with anthropogenic interventions, and possibly due to the application of inorganic fertilizers at the South African sites.

The C/N ratio of the surface soils varied between 9.3 and 10.3 at Nandi, from 8.7 to 11.6 at Kakamega, from 8.9 to 11.8 at the South African sites. Unlike the total SOC and N concentrations, however, there was no consistent trend observed in C/N ratio with the duration of cultivation following the conversion of the native forest and grassland sites into agricultural fields. We attribute this to a highly significant correlation between the losses of SOC and N with time in the forest-derived soils of Kenya ($r = 0.99$ at Nandi and $r = 0.98$ at Kakamega) and grassland-derived soils of South Africa ($r = 0.99$), indicating the close coupling in loss kinetics of these two parameters in the investigated soils. These results partly agree with the results of Dalal & Mayer (1986b) who observed that cultivation had no significant effect on the C/N ratio of some Australian soils. However, they contradict those of Haas *et al*. (1957) who reported lower C/N ratio in cultivated than virgin soils in the Great Plains, as losses of SOC exceeded that of N following conversion of native grassland sites to agricultural fields.

Elemental compositions and ratios of humic fractions

Elemental analysis provides information on the distribution of major elements in humic fractions and can be used as an important indicator of the degree of decomposition. The data in Table 2 shows that the average proportions of SOC (42.1%, 41.3% and 37.1%), N (3.9%, 3.9% and 3.3%), H (4.9%, 4.5% and 4.3%) and O (39.5%, 38.9% and 37.5%) in the humic fractions extracted from the forest-derived soils of Nandi and Kakamega and grassland-derived soils of South Africa, respectively. These values are within the ranges reported for humic fractions extracted from tropical soils by Schnitzer & Khan (1978), Stevenson (1982) and Pérez *et al*. (2004). Our results indicate relatively lower proportions of organic C, N and H and a slightly higher proportion of O in the humic fractions extracted from soils under long-term cropping compared with those from native forest and grassland soils. The trends in the present investigation compare favorably with the patterns observed by Martin *et al*. (1998), who showed that cultivation usually decreases the contents of C, N and H but increases O concentration due to oxidative degradation of humic fractions.

The ratio of C/H has been used as an aromaticity index, where a larger C/H value is associated with a higher degree aromaticity in humic fractions (McDonnell *et al*., 2001). Our elemental ratios showed that land-use changes slightly increased the ratio of C/H and that alkali extractable SOM in the cultivated soils appear to be more aromatic compared with the native sites. There was no consistent trend observed in the C/N ratio of the humic fractions. However, the results in Table 2 indicates that the O/H ratio increased, while the C/O ratio tend to decrease with time following the conversion of the forests and grasslands to continuous cultivation in the humic fractions. These results suggest increased biological oxidation associated with decrease in readily mineralizable organic C biomolecules such as some carbohydrates, amino acids and amino sugars in the cultivated soils as a result of land clearing and mechanical disturbance of the soil by tillage practices (Zhang *et al*., 1999; Solomon *et al*., 2000, 2002; Brodowski *et al*., 2004).

Organic C speciation and long-term dynamics using ^{13}C NMR spectroscopy

The stacked ^{13}C NMR spectra of the humic fractions extracted from the bulk soils of Kenyan and South African sites (Fig. 2) revealed multiple peaks between $\delta = 0$ and 50 ppm indicating highly heterogeneous composition of alkyl-C species due to the presence of (poly)-methylene chains and branched aliphatic domains such

Table 2 Elemental composition and ratios of humic fraction extracts of soils from the long-term chronosequences of Kenya and South Africa

Site	Land use	% C	% N	% H	% O	C/N	C/H	C/O	O/H
Kenya									
Nandi									
	Natural forest	43.2	4.0	5.1	38.4	10.8	8.4	1.12	7.5
	2 years cultivated	43.9	3.8	4.6	40.4	11.5	9.5	1.09	8.8
	4 years cultivated	43.1	4.0	4.8	40.2	10.8	9.0	1.07	8.4
	20 years cultivated	41.3	4.0	4.8	39.2	10.3	8.7	1.05	8.3
	30 years cultivated	41.1	4.1	5.0	39.1	10.1	8.2	1.05	7.8
	50 years cultivated	41.1	4.2	5.1	39.9	9.7	8.1	1.03	7.8
	80 years cultivated	41.3	3.8	4.9	38.1	11.0	8.4	1.08	7.7
	100 years cultivated	41.6	3.5	4.6	40.9	12.0	9.1	1.02	8.9
Kakamega									
	Natural forest	43.6	4.0	4.8	38.8	10.9	9.0	1.12	8.0
	2 years cultivated	43.6	4.1	5.0	38.4	10.6	8.7	1.14	7.6
	4 years cultivated	44.1	3.9	4.3	38.3	11.3	10.2	1.15	8.9
	18 years cultivated	40.7	4.3	4.3	39.1	9.5	9.4	1.04	9.1
	45 years cultivated	39.6	4.1	4.6	37.9	9.6	8.6	1.05	8.3
	73 years cultivated	39.1	3.4	4.1	41.2	11.7	9.6	0.95	10.1
	100 years cultivated	38.6	3.8	4.1	38.9	10.4	9.4	0.99	9.5
South Africa	Natural grassland	38.4	3.5	4.5	37.2	11.01	8.5	1.03	8.2
	3 years cultivated	37.2	3.3	4.1	37.1	11.4	9.0	1.00	9.0
	8 years cultivated	37.5	3.3	4.2	37.6	11.5	8.9	1.00	8.9
	10 years cultivated	36.6	3.2	4.1	38.0	11.5	8.9	0.96	9.3
	20 years cultivated	34.6	3.1	4.6	37.0	11.2	7.6	0.93	8.1
	30 years cultivated	36.7	3.2	4.0	36.7	11.6	9.2	1.00	9.1
	40 years cultivated	37.7	3.3	4.1	36.7	11.4	9.2	1.03	9.0
	60 years cultivated	36.3	3.3	4.4	38.7	10.9	8.3	0.94	8.8
	90 years cultivated	38.6	3.4	4.4	38.2	11.2	8.7	1.01	8.6

as those found in lipids, hemicellulose, proteins, aliphatic biopolymers such as cutin and suberin (Skjemstad *et al*., 1983). The resonances between $\delta = 50$ and 60 ppm are attributed to methoxyl-C groups indicatives of syringyl and guaiacyl (sinapyl) units of lignin, as well as to N-alkyl C from polypeptides (Kögel-Knabner, 2002). Chemical shifts between $\delta = 60$ and 110 ppm represent mainly O-alkyl-C structures attributed to diverse group of carbohydrates that produce signals between $\delta = 60$ and 95 ppm due to the presence of C2 to C6 structures such as cellulose, hemicellulose and other polymeric carbohydrates, as well as alcohols and ether-bonded aliphatic C; while signals between $\delta = 95$ and 110 ppm are ascribed to anomeric C (C1) polysaccharides and ketals (Kögel-Knabner, 2002; Jokic *et al*., 2003). Aromatic-C resonated between $\delta = 110$ and 165 ppm, where the broad signals between $\delta = 110$ and 145 ppm are attributed to a wide variety of H- and C-substituted aryl-C structures, while peaks between $\delta = 145$ and 165 ppm arise from O-substituted aryl C indicative of lignin-derived phenols such as syringyl, guaiacyl and *p*-hydroxyphenyl structures (Kögel-Knabner, 2002). Chemical shifts between $\delta = 165$ and 220 ppm are assigned to carbonyl-C groups representing a variety organic C functionalities such as carboxylic acid, amide and polypeptides ($\delta = 165$–175 ppm), whereas signals between $\delta = 185$ and 220 ppm are attributed to aldehyde-C, ketonic-C and quinone-C (Skjemstad *et al*., 1983).

The relative proportion of organic C functional groups identified by ^{13}C NMR spectroscopy (Table 3) indicate that structures containing O-alkyl-C are the dominant organic C forms representing on average for 37.7%, 38.0% and 35.3% of the total SOC identified by ^{13}C NMR spectroscopy at the Nandi, Kakamega and the South African sites, respectively. Aromatic-C (H- and C-substituted aryl-C and O-aryl-C) structures were the second most abundant organic C species constituting on average for 22.0%, 21.9% and 25.6% of the total SOC at the three sites, respectively. Alkyl-C represented 16.1% at Nandi, 15.5% at Kakamega and 16.8% at the South African sites; whereas carbonyl-C accounted for 13.9%, 14.8% and 16.5%, respectively. Methoxyl-C and N-alkyl-

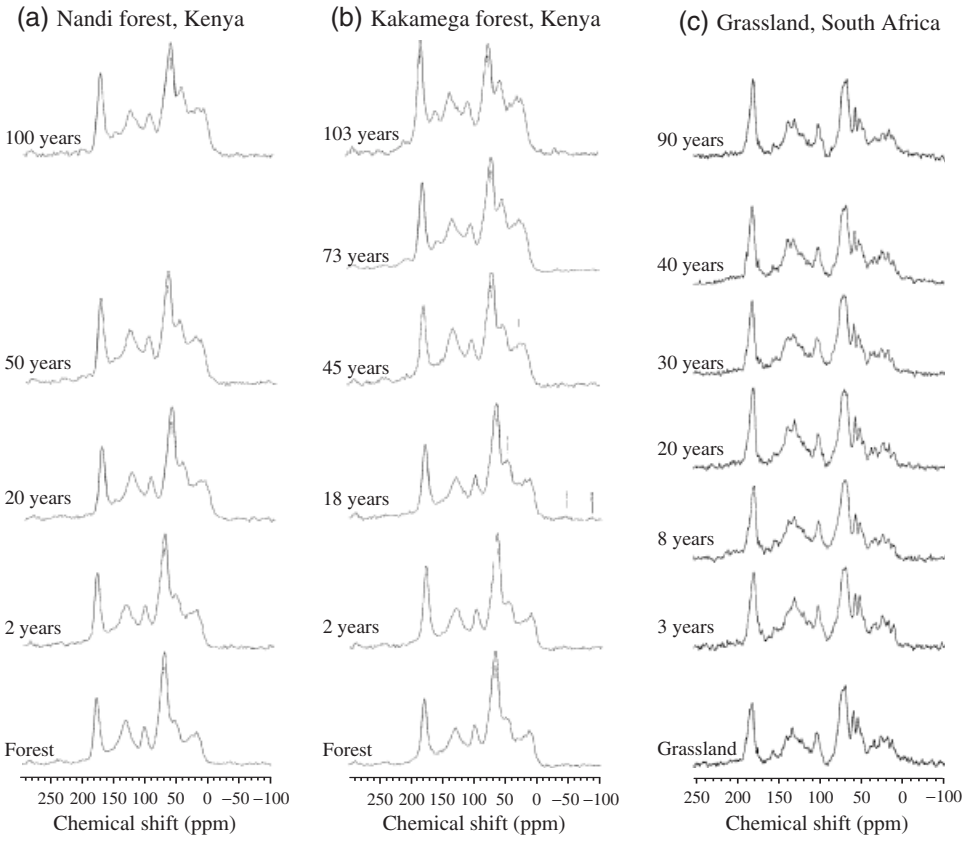

Fig. 2 Stacked ^{13}C N nuclear magnetic resonance spectra of humic fractions from the long-term chronosequences of Kenya and South Africa.

C accounted only for smaller portion (10.2%, 10.0% and 5.8%) of the total SOC identified by ^{13}C NMR in the humic fractions extracted from the three agroecosystems (Table 3).

Integration of signal intensities resolved by ^{13}C NMR spectroscopy (Table 3) indicated subtle but consistent differences in the structural composition of SOC in the humic fractions following land-use and land-cover changes. Clearing natural vegetation and subsequent cultivation resulted in relatively smaller proportions of O-alkyl-C structures (from 39.8% to 35.6% at Nandi, from 42.0% to 34.2% at Kakamega and from 38.0% to 33.8% at the South African sites) in the humic fractions. However, with the exception of native grassland soils of South Africa where higher proportions of alkyl-C were detected by NMR, small but consistent increases in proportions of alkyl-C and aromatic-C structures were observed in the humic fractions following land-use and land-cover changes. The trends for carbonyl-C and methoxyl-C structures were not consistent.

As the concomitant decrease of labile organic C structures and increase of recalcitrant components of SOC move in opposite directions as decomposition proceeds, Baldock *et al*. (1997) suggested that ratios of various organic C functionalities may provide sensitive indices of the extent of decomposition of SOM. Hence, we applied the following indexes: O-alkyl-C to aromatic-C, aromatic-C to alkyl-C + methoxyl-C + O-alkyl-C + aromatic-C + carbonyl-C (aromaticity), and alkyl-C to O-alkyl-C to further evaluate the extent of changes in the structural composition of SOC in the humic fractions with time following land-use changes. Clearing the natural vegetation and converting it in to long-term continuous cultivation resulted in a gradual decrease of O-alkyl-C to aromatic-C ratio from 1.8 to 1.5 at Nandi, from 2.2 to 1.4 at Kakamega and from 1.8 to 1.4 at the South African sites (Table 3), while the degree of aromaticity and aliphaticity (alkyl-C to O-alkyl-C) tend to show an increasing tendency in the arable soils with the duration of cultivation. These trends agree with the changes observed in structural composition of SOC determined using ^{13}C NMR spectroscopy in other tropical soils following land-use transitions by Golchin *et al*. (1995), Baldock *et al*. (1997), Skjemstad *et al*. (2001) and Solomon *et al*. (2002). However, the ratio of alkyl-C to O-alkyl-C in the native grassland soils of

Table 3 Relative proportions of organic C functional groups in the humic fractions resolved by ^{13}C NMR spectroscopy

Site	Land use	%					O-alkyl-C/ Aromatic-C	Aromaticity†	Alkyl-C/ O-alkyl-C
		Alkyl	Methoxyl	O-Alkyl-C	Aromatic*	Carbonyl			
Kenya									
Nandi									
	Forest	13.9	9.1	39.8	22.2	15.0	1.79	0.22	0.35
	2 years cultivated	15.3	10.0	39.0	21.6	14.1	1.81	0.22	0.39
	20 years cultivated	16.5	10.5	37.9	22.1	13.0	1.72	0.22	0.43
	80 years cultivated	18.6	11.3	36.3	20.6	13.2	1.76	0.21	0.51
	100 years cultivated	16.4	10.1	35.6	23.6	14.3	1.51	0.24	0.46
Kakamega									
	Forest	13.3	9.3	42.0	19.0	16.4	2.20	0.19	0.32
	2 years cultivated	15.2	9.5	40.0	21.9	13.4	1.82	0.22	0.38
	18 years cultivated	15.9	10.7	39.3	19.9	14.2	1.98	0.20	0.40
	45 years cultivated	15.1	10.0	37.0	23.7	14.2	1.56	0.24	0.41
	73 years cultivated	16.6	10.8	35.3	22.5	14.8	1.57	0.23	0.47
	103 years cultivated	16.6	9.4	34.2	24.2	15.6	1.41	0.24	0.48
South Africa	Grassland	19.8	5.0	38.0	21.5	15.7	1.77	0.21	0.52
	3 years cultivated	16.3	8.2	34.2	27.6	13.7	1.24	0.28	0.48
	10 years cultivated	14.6	3.7	36.8	27.4	17.5	1.34	0.27	0.40
	20 years cultivated	9.1	10.7	35.4	27.0	17.8	1.31	0.27	0.26
	40 years cultivated	16.6	4.2	37.6	25.7	15.9	1.46	0.26	0.44
	60 years cultivated	20.4	4.5	31.5	26.4	17.2	1.19	0.26	0.65
	90 years cultivated	20.6	4.3	33.8	23.5	17.8	1.44	0.23	0.61

*Aromatic, O-aryl-C + H- and C-substituted aryl-C.
†Aromaticity, aromatic-C/alkyl-C + methoxyl-C + O-alkyl-C + aromatic-C + carbonyl-C.
NMR, nuclear magnetic resonance.

South Africa was still high compared with most agricultural fields and does not conform to the above trend. Abrupt changes in the progressive increase in alkyl-C to O-alkyl-C ratios have also been observed by Golchin *et al*. (1995), and attributed to selective stabilization of organic inputs or metabolic products of aliphatic nature by adsorption to mineral surfaces and formation of organo-mineral complexes in native soils. Despite the overall similarity of the spectra, which might be ascribed to the normally low signal recovery, the fine scale shifts in chemical composition evident from our ^{13}C NMR spectra and the trends observed in the decomposition indexes indicate losses of more readily decomposable O-alkyl-C structures such as polysaccharides from the humic fractions and an accumulation of more recalcitrant aromatic-C and alkyl-C structures during the course of decomposition processes following long-term anthropogenic land-use and land-cover changes.

Organic C speciation and long-term dynamics using Sr-FTIR-ATR spectroscopy

Sr-FTIR-ATR spectra of humic fractions contain a variety of bands that are diagnostic and can serve as a valuable tool not only to characterize the principal classes of chemical groups of which SOM is comprised but also to investigate management induced changes in the composition of SOM (Solomon *et al*., 2005b). The Sr-FTIR-ATR spectra recorded from the humic fractions in the present experiment displayed characteristic absorption band patterns in the frequency range of 4000–800 cm^{-1} indicating the presence of extremely heterogeneous structures in these forest- and grassland-derived soils (Fig. 3). A strong broad band at about 3370 cm^{-1} represents stretching vibrations of H-bonded hydroxyl (O–H) groups of phenols with traces of amine (N–H) stretch (Stevenson, 1982). The weak vibrations at about 2920 and 2853 cm^{-1} represent asymmetric and symmetric aliphatic-C (CH_3 and CH_2) stretchings. These peaks are intense and much better resolved in the humic fractions extracted from the grassland-derived soils of South Africa compared with the humic fractions extracted from the forest-derived soils of Kenya. The slight shoulder around 1720 cm^{-1} (only visible in the forest-derived soils) is due to the C=O stretching of carboxylic-C and ketonic-C. The pronounced broad band at about 1642 cm^{-1} is mainly attributed to aromatic-C (C=C) vibrations and to a smaller extent to

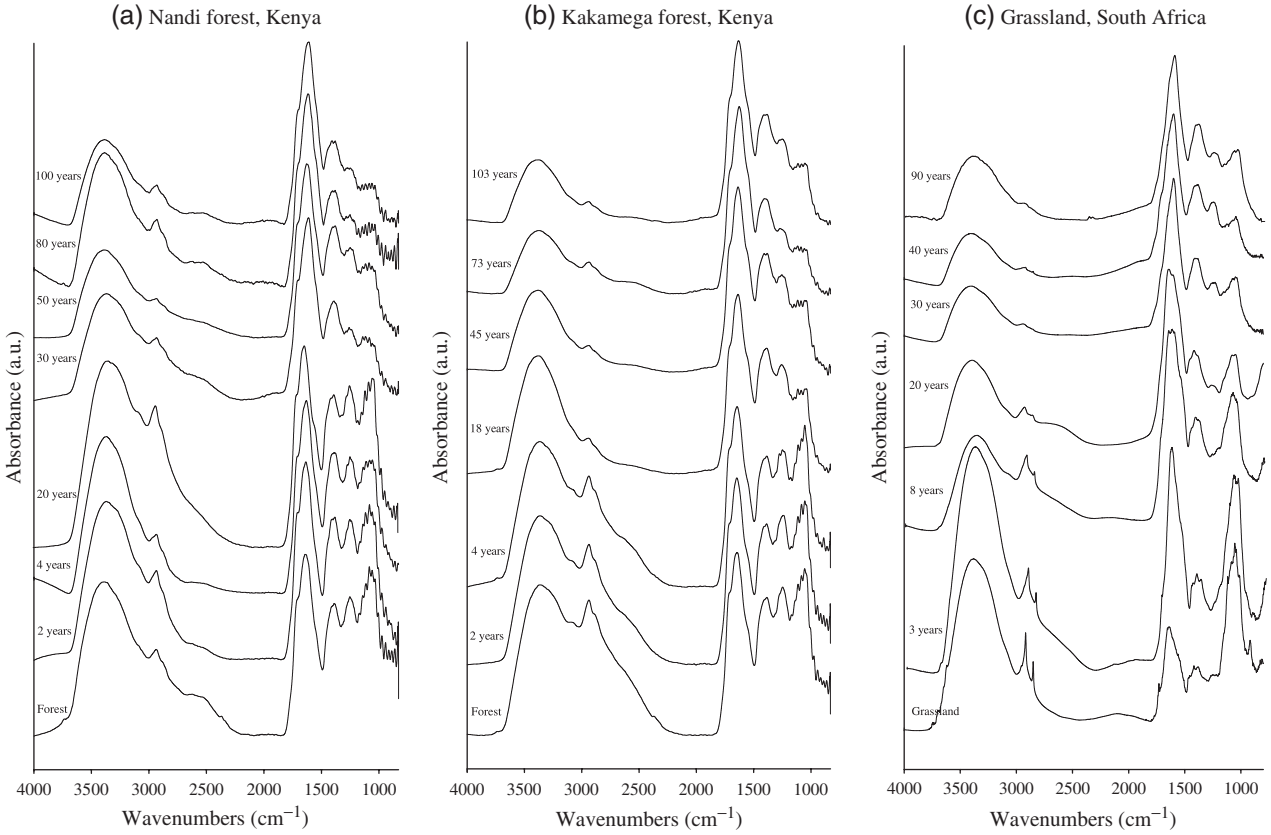

Fig. 3 Synchrotron-based Fourier transform infrared-attenuated total reflectance spectra of humic fractions from the long-term chronosequences of Kenya and South Africa.

C=O stretching in quinones and ketonic acids (Stevenson, 1982; Solomon *et al*., 2005b). The band which appeared around 1389 cm^{-1} arises from aliphatic (C–H) deformation of CH_2 or CH_3 groups (Stevenson, 1982). The band around 1252 cm^{-1} could be attributed to C–O stretching and OH deformation of carboxylic-C (COOH) groups (Lehmann *et al*., 2005; Solomon *et al*., 2005b). The strong band at about 1071 cm^{-1} originated from C–O stretching vibrations of polysaccharides (Stevenson, 1982; Solomon *et al*., 2005b).

Relative proportions of organic C functional groups calculated from peak intensities of Sr-FTIR-ATR spectra of the humic fractions show that aromatic-C constitute on average for 27.0%, 27.5% and 28.3% of the total SOC identified by this technique, followed by almost similar proportions of H-bonded O–H groups of phenols (18.3%, 15.6% and 14.4%) and aliphatic-C deformation of CH_2 or CH_3 (16.6%, 17.9% and 15.4%) at Nandi, Kakamega and the South African sites, respectively (Table 4). The C–O stretching and OH deformation of carboxylic-C (COOH) groups accounted for 14.5%, 15.4% and 12.7%, while C–O stretching vibrations of polysaccharide-C represented on average 13.9%, 14.8% and 17.1% of the total SOC, respectively. The proportion of asymmetric and symmetric aliphatic-C groups amounted on average only to 9.7%, 8.8% and 12.2% of the total SOC identified by Sr-FTIR-ATR spectroscopy in the humic fractions extracted from the forest-derived soils of Nandi, Kakamega and grassland-derived soils of South Africa, respectively.

Sr-FTIR-ATR spectra (Fig. 3) and relative proportions of signal intensities (Table 4) clearly demonstrated the apparent impact of anthropogenic perturbations on the structural composition of SOC at the molecular level in these tropical and subtropical agroecosystems. The most prominent changes in signal intensities appeared near 1071 and 1626 cm^{-1} regions (Fig. 3). The relative proportions of C–O stretching vibrations of polysaccharides decreased from 19.4% to 8.5% at Nandi, from 19.7% to 9.2% at Kakamega and from 25.4% to 13.9% at the South African sites following land-use changes. In contrast, the relative proportion of aromatic-C (C=C) vibrations increased from 21.1% to 36.9% at Nandi, from 18.8% to 38.3% at Kakamega and from 14.3% to 33.3% at the South African sites, indicating that more recalcitrant aromatic-C forms are becoming the domi-

Table 4 Relative proportions of the different organic C species in humic fractions resolved by SR-FTIR-ATR spectroscopy

		%					
Site	Land use	Phenolic-C	Aliphatic-C*	Aromatic-C	Aliphatic-C†	Carboxylic-C	Polysaccharide-C
Kenya							
Nandi							
	Forest	17.9	10.1	21.1	15.4	16.1	19.4
	2 years cultivated	18.0	10.1	22.4	16.2	15.9	17.4
	4 years cultivated	19.2	7.2	23.6	17.2	16.6	16.2
	20 years cultivated	18.4	14.0	19.9	15.1	15.9	16.8
	30 years cultivated	15.3	6.8	30.2	19.4	15.6	12.7
	50 years cultivated	18.3	10.7	31.2	17.0	12.3	10.5
	80 years cultivated	21.5	10.8	31.0	15.4	11.5	10.0
	100 years cultivated	17.4	8.3	36.9	17.1	11.8	8.5
Kakamega							
	Forest	14.4	14.70	18.80	15.0	17.4	19.7
	2 years cultivated	20.4	13.20	20.50	15.5	11.5	18.9
	4 years cultivated	16.8	12.90	20.90	15.1	15.8	18.5
	18 years cultivated	18.1	5.40	27.50	19.2	16.7	13.1
	45 years cultivated	14.1	4.70	31.70	20.4	16.7	12.4
	73 years cultivated	12.2	4.30	34.90	20.9	16.2	11.5
	103 years cultivated	13.0	6.60	38.30	19.5	13.4	9.2
South Africa	Grassland	17.6	20.2	14.3	8.8	13.7	25.4
	3 years cultivated	19.5	17.8	22.8	9.4	11.1	19.4
	10 years cultivated	12.7	17.5	26.6	15.0	10.1	18.1
	20 years cultivated	14.8	12.4	30.2	16.4	10.6	15.6
	40 years cultivated	12.6	4.1	36. 9	18. 9	13.3	14.2
	60 years cultivated	10.2	6.2	33.8	19.9	16.5	13.4
	90 years cultivated	12.9	6.9	33.3	19.4	13.6	13.9

*Aliphatic-C vibration of asymmetric and symmetric CH_3 and CH_2 groups.
†Aliphatic-C deformation of CH_2 or CH_3 groups.
SR-FTIR-ATR, synchrotron-based Fourier transform infrared-attenuated total reflectance.

nant forms of organic C functional groups in the humic fractions extracted from the cultivated soils. The peak intensities of Sr-FTIR-ATR spectra also indicated small but consistent increases in aliphatic (C–H) deformation of CH_2 or CH_3 groups following land-use and land-cover changes signifying accumulation of some recalcitrant aliphatic structures in the arable soils. Compared with aliphatic (C–H) deformation of CH_2 or CH_3 groups, signal intensities of asymmetric and symmetric aliphatic (CH_3 and CH_2) vibrations that resonate at about 2920 and 2853 cm^{-1} showed an opposite trend, whereby relative proportions of these organic C functionalities decreased following anthropogenic interventions. These results are in accordance with our previous investigation on the fate of aliphatic-C structures in humic fractions extracted from tropical soils (Solomon *et al*., 2005b). Similar to ^{13}C NMR spectroscopy, Sr-FTIR-ATR spectra recorded from the humic fraction extracts of the native grassland soils of South Africa indicated the presence of higher asymmetric and symmetric aliphatic-C, which could explain the larger total alkyl-C structures and the unusually high alkyl-C to O-alkyl-C ratios obtained from these sites. The changes in relative proportions of C–O stretching and OH deformation of carboxylic (COOH) groups and H-bonded hydroxyl (O–H) groups of phenols were small and inconsistent in both forest- and grassland-derived soils. These results are supported by the trends observed from O-alkyl-C to aromatic-C, aromaticity and aliphaticity indexes (Fig. 4). According to Fig. 4a, the ratio of O-alkyl-C to aromatic-C decreased considerably with time in the humic fractions (from 0.92 to 0.23 at Nandi, from 1.1 to 0.24 at Kakamega and from 1.8 to 0.42 at the South African sites) after clearing and subsequent establishment of cultivated fields. However, the degree of aromaticity (from 0.21 to 0.36 at Nandi, from 0.18 to 0.49 at Kakamega and from 0.15 to 0.36 at the South African sites; Fig. 4b) and aliphaticity (from 1.3 to 3.0 at Nandi, from 1.5 to 2.8 at Kakamega and from 1.1 to 1.9 at the South African sites; Fig. 4c) of the humic fractions increased with time due to transitions from natural forest and grassland ecosystems to arable land

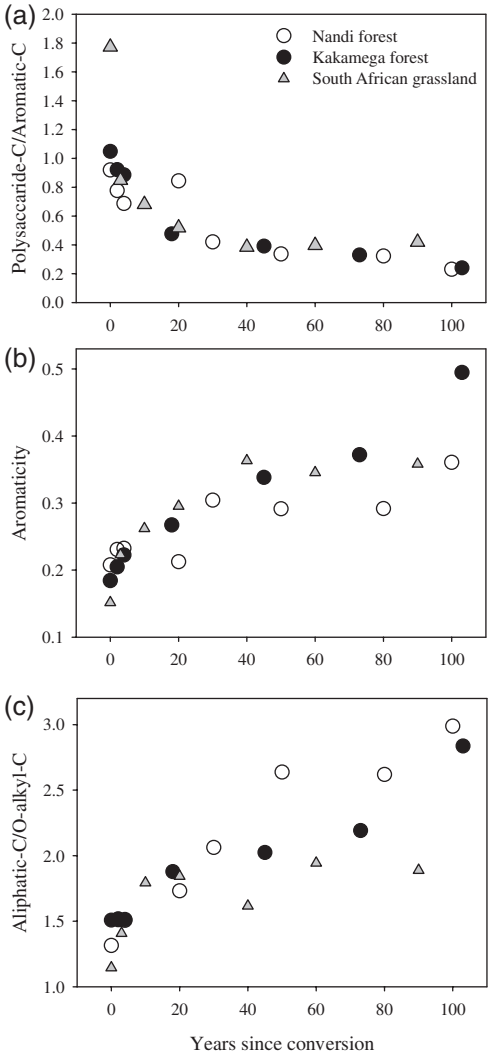

Fig. 4 Ratio of polysaccharide-C to aromatic-C (a), aromaticity (b) and aliphatic-C to polysaccharide-C (c) from the tropical forest agroecosystems of Kenya (Nandi and Kakamega) and subtropical grassland agroecosystems of South Africa using synchrotron-based Fourier transform infrared-attenuated total reflectance spectroscopy.

(Fig. 4b and c). The Sr-FTIR-ATR spectral patterns, proportions and ratios of the humic fractions clearly indicate that easily degradable SOM constituents, such as polysaccharide-C and some aliphatic-C moieties were rapidly oxidized due to accelerated mineralization following land clearing and long-term cultivation. Therefore, it is possible to suggest that structural composition of the SOM in the agriculturally managed soils was considerably different from the SOM in the natural forests and grassland ecosystems, and was dominated by more recalcitrant forms of aromatic-C and aliphatic-C structures. Our results also provide evidence that

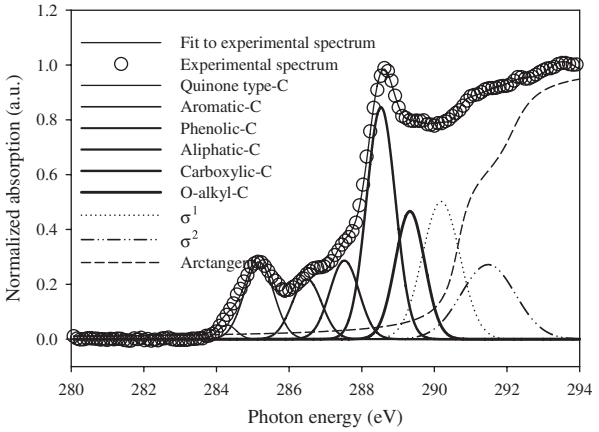

Fig. 5 Speciation of soil organic carbon (SOC) using typical C (1s) near-edge X-ray absorption fine structure spectra deconvolution showing the main 1s–π^* and 1s–3p/σ^* transitions, two σ^* transitions and arctangent step functions.

changes in functional group chemistry of the resultant SOC due to anthropogenic interventions continued long after new steady-state equilibrium of total SOC (t_e) has been approached in these tropical and subtropical agroecosystems. The changes in the inherent SOM structures influence the biogeochemical cycling of C and bioavailability of the nutrients associated with SOM.

Organic C speciation and long-term dynamics using C (1s) NEXAFS spectroscopy

Energy positions of the main 1s–π^* transitions in the fine structure regions (284–290 eV) of the deconvoluted C (1s) NEXAFS spectra (Fig. 5) show characteristic descript functional groups (Solomon *et al.*, 2005b), which allowed us to fingerprint the various SOC structures present in the humic fractions. They also provide a means for semiquantitative comparison of organic C species present in the humic fractions at the various levels of decomposition, which seems to be a promising way to obtain a deeper insight into the structural composition and turnover dynamics of organic C in terrestrial ecosystems (Scheinost *et al.*, 2001; Schäfer *et al.*, 2003; Solomon *et al.*, 2005b). The C 1s–π^* transition near 284.4 eV (G1) is associated with quinone type-C, such as benzoquinone and with protonated and alkylated aromatic-C or heteroatom-substituted aromatics (Schäfer *et al.*, 2003, 2005; Solomon *et al.*, 2005b). The resonance near 285.2 eV (G2) is assigned to aromatic-C (protonated and alkylated to carbonyl-substituted aromatic-C) and possibly olefinic-C (Cody *et al.*, 1998; Ade & Urquhart, 2002; Lehmann *et al.*, 2005). The sum of G1 and G2 is used in subsequent discussions throughout this paper to represent total aromatic-C.

Table 5 Relative proportions of organic C functional groups in humic fractions identified by C (1s) NEXAFS spectroscopy

Site	Land use	% Quinine	Aromatic	Phenolic	Aliphatic	Carboxylic	O-alkyl-C	O-alkyl-C/ Aromatic-C	Aromaticity*	Alkyl-C/ O-alkyl-C
Kenya										
Nandi										
	Forest	5.1	11.9	15.0	10.1	35.0	22.9	1.35	0.17	0.44
	2 years	4.1	13.3	15.7	12.1	32.5	22.4	1.29	0.17	0.54
	4 years	0.0	18.1	8.1	3.4	49.5	20.9	1.15	0.18	0.16
	20 years	2.3	14.6	9.5	8.1	42.2	23.3	1.38	0.17	0.35
	30 years	4.6	15.1	13.0	10.8	33.3	23.3	1.18	0.20	0.46
	50 years	5.4	17.2	13.7	11.3	32.8	19.6	0.87	0.23	0.58
	80 years	1.6	15.2	9.6	7.6	40.7	25.5	1.52	0.17	0.30
	100 years	5.1	15.8	13.5	12.2	31.7	21.7	1.04	0.21	0.56
Kakamega										
	Forest	4.7	10.7	14.9	10.7	35.4	23.5	1.52	0.15	0.45
	2 years	4.8	14.3	16.5	13.0	28.5	22.9	1.20	0.19	0.57
	4 years	5.8	11.1	12.5	11.3	35.1	24.3	1.44	0.17	0.47
	18 years	3.1	11.0	9.5	8.1	39.1	29.2	2.07	0.14	0.28
	45 years	3.4	13.7	10.2	8.9	39.4	24.4	1.43	0.17	0.36
	73 years	1.7	14.7	9.6	8.3	42.2	23.4	1.43	0.16	0.35
	103 years	8.8	9.6	2.0	0.50	57.8	21.3	1.15	0.18	0.02
S. Africa†	Grassland	2.2	13.3	13.7	13.1	32.2	25.5	1.64	0.16	0.52
	3 years	2.3	18.9	14.9	11.3	33.5	19.1	0.90	0.21	0.59
	10 years	1.7	14.4	11.1	14.6	38.7	19.6	1.22	0.16	0.74
	20 years	0.0	15.3	10.0	15.6	40.0	19.1	1.25	0.15	0.81
	40 years	1.6	13.5	10.4	13.6	39.1	21.8	1.45	0.15	0.62
	60 years	2.2	13.6	10.9	18.0	31.3	23.9	1.51	0.16	0.75
	90 years	0.0	22.1	16.7	17.6	21.1	22.5	1.02	0.22	0.78

*Aromaticity, total aromatic-C/quinine-type-C + aromatic-C + phenolic-C + aliphatic-C + carboxylic-C + O-alkyl-C.
†S. Africa, South Africa.

The absorption band near 286.5 eV (G3) corresponds primarily to phenolic-C including O-substituted aryl-C indicative of lignin and possibly to Ketonic-C and phenyl-C attached to amide group (Cody *et al*., 1998; Rothe *et al*., 2000; Ade & Urquhart, 2002; Schäfer *et al*., 2003). The 1s–3p/σ* transitions near 287.3 eV (G4) is due to aliphatic-C of CH_3, CH_2 and CH nature, while the strong resonance near 288.4 eV (G5) represent C 1s–π* transitions of carboxylic-C (Cody *et al*., 1998). The signal near 289.3 eV (G6) is attributed to C 1s–π* transitions of O-alkyl-C group representing mainly polysaccharides, as well as smaller proportions of alcohol and ether-C (Scheinost *et al*., 2001; Solomon *et al*., 2005b).

The stacked C K-edge NEXAFS spectra (Fig. 6) and the relative proportions of organic C forms obtained by spectral deconvolution of the humic fractions (Table 5) indicate that C 1s–π* transitions of carboxylic-C are the most prominent forms of organic C functional groups. They accounted on average for 37.2%, 39.7% and 33.7% of the total organic C determined by C (1s) NEXAFS spectroscopy of the humic fractions extracted from Nandi, Kakamega and the South African soils, respectively. O-alkyl-C structures are the second most dominant organic C forms representing on average 22.4%, 24.1% and 21.6% of the total SOC followed by aromatic-C structures (18.7%, 16.8% and 17.3%) at the three sites, respectively. Our results are in agreement with C K-edge NEXAFS results of Rothe *et al*. (2000), Schäfer *et al*. (2003) and Solomon *et al*. (2005b), where the electronic transitions corresponding to carboxylic-C and O-alkyl-C structures in humic fractions were found to be the dominant forms of organic C moieties. They also compare favorably with NEXAFS spectra taken directly from the surface layers soils by Jokic *et al*. (2003), where large proportions of O-alkyl-C and carboxylic-C followed by aromatic-C structures have been observed. The average proportions of C 1s–π* transitions of phenolic-C constituents extracted from the forest-derived soils were generally higher (12.3% at Nandi, 10.7% at Kakamega) than the aliphatic-C (9.5% at Nandi, 8.7% at Kakamega) structures (Table 5). Unlike the forest-derived soils, relatively more C 1s–3p/σ* transitions of aliphatic-C (14.8%) were found compared with phenolic-C constituents (12.5%) in the humic fractions ex-

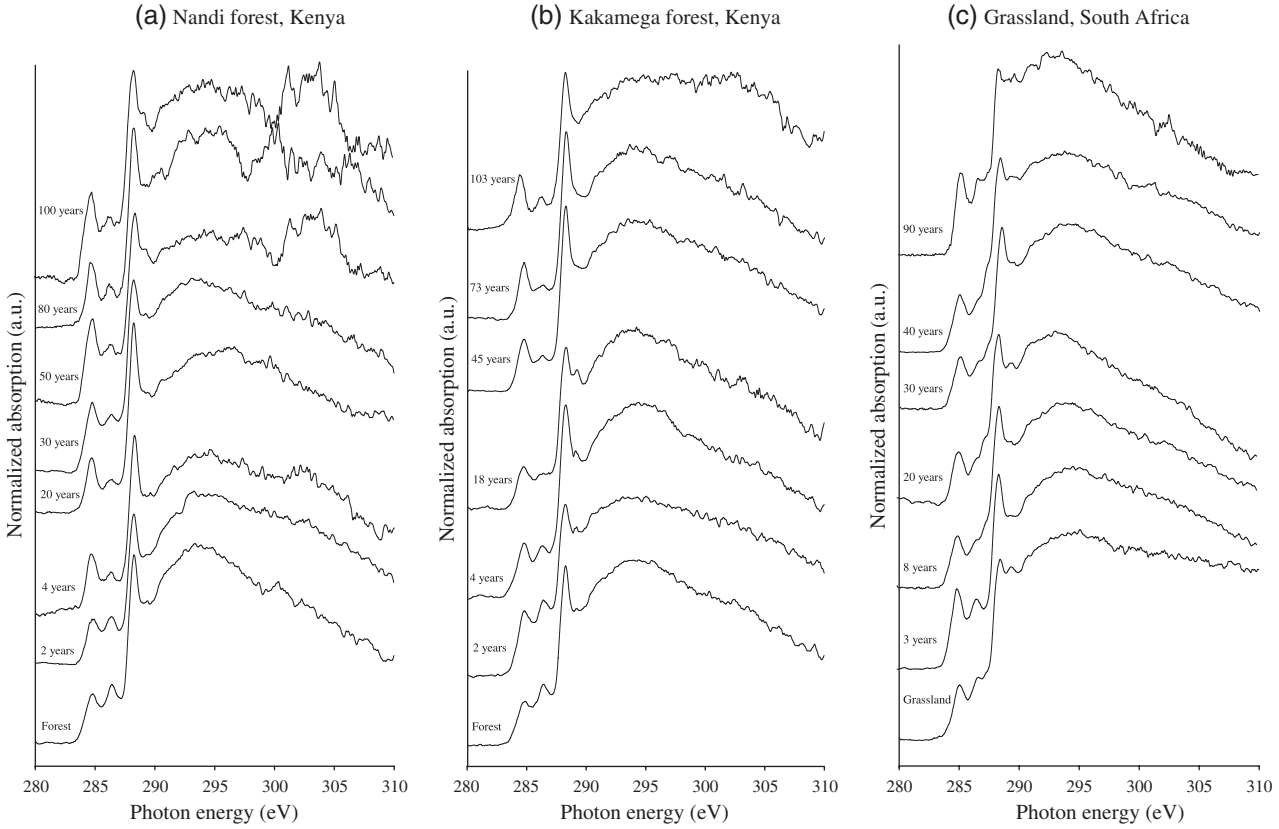

Fig. 6 Stacked C (1s) spectra of humic fractions from the long-term chronosequences of Kenya (Nandi and Kakamega) and South Africa.

tracted from the grassland-derived soils of South Africa. These results complement our observations of aliphatic-C functionalities isolated using ^{13}C NMR and Sr-FTIR-ATR spectroscopy of humic fractions extracted from the native grassland soils of South Africa.

Examination of C (1s) NEXAFS spectral features revealed that anthropogenic land-use and land-cover changes considerably influenced the spectral features of the humic fractions extracted from the soils under investigation (Fig. 6). The most prominent qualitative changes in C (1s) NEXAFS spectra occurred near 285 eV. As can be noted from the stacked spectra in Fig. 6, the changes in signal intensity of C 1s–π^* aromatic-transitions appeared as early as the fourth year after conversion and their intensity continued to increase with the duration of cultivation well after the new steady state was approached in all the three agroecosystems. These changes were also reflected by the relative proportions of aromatic-C transitions and by the degree of aromaticity (Table 5). The proportions of aromatic-C increased from 17.0% to 20.9% at Nandi, from 15.4% to 18.4% at Kakamega and from 15.5% to 22.1% at the South African sites. We also noted other subtle but salient qualitative and quantitative changes following anthropogenic interventions such as the decrease in resonances of the absorption bands at about 286.5 eV that corresponds to phenolic-C including O-substituted aryl-C indicative of lignin, and at about 289.3 eV representing 1s–π^* transitions of O-alkyl-C group composed mainly of polymeric carbohydrates (Fig. 6). Oxidative degradation of lignin-derived phenols following cultivation was also observed from the South African sites by Lobe *et al*. (2002). The changes in O-alkyl-C functional groups were reflected by the decreasing trends O-alkyl-C to aromatic-C ratios (from 1.4 to 1.0 at Nandi, from 1.5 to 1.2 at Kakamega and from 1.6 to 1.0 at the South African sites) with an increase in duration of cultivation (Table 5). In contrast, alkyl-C to O-alkyl-C ratio of the humic fractions extracted from the forest-derived soils of Nandi (from 0.44 to 0.56) and grassland-derived soils of South Africa (from 0.52 to 0.78) increased with the duration of intervention. The trends observed at the Kakamega site for this ratio were not consistent. The C (1s) NEXAFS spectral features, relative proportions and ratios of the various organic C functionalities in general reflect: (i) the continued degradation and loss of O-alkyl structures such as those found in carbohydrates or in cellulose, side-chain oxi-

dations of phenylpropane unit of lignin structures with time, and (ii) the continual aromatization and aliphatization of the SOC possibly due to accumulation of a wide variety of recalcitrant H- and C-substituted aryl-C structures and aliphatic components such as (poly)-methylene units during the decomposition process following long-term anthropogenic perturbations. These observations complement the results obtained by elemental analysis, as well as by ^{13}C NMR and Sr-FTIR-ATR spectroscopic techniques.

Relationship of C functionalities identified by the different spectroscopic techniques

Although C (1s) NEXAFS spectroscopy has been used to fingerprint the molecular structures of extremely heterogeneous organic compounds, such as humic fractions (Rothe *et al*., 2000; Scheinost *et al*., 2001; Schäfer *et al*., 2005; Solomon *et al*., 2005b) as well as for probing surficial (Jokic *et al*., 2003) and spatial (Lehmann *et al*., 2005) C functional group heterogeneity of mineral soils and black C particles, respectively, only a few studies have investigated the relationships between C forms identified by C K-edge NEXAFS and ^{13}C NMR spectroscopy (Scheinost *et al*., 2001; Schäfer *et al*., 2003; Solomon *et al*., 2005b). The correlations between organic C functional groups identified by C (1s) NEXAFS and Sr-FTIR-ATR spectroscopy of humic fractions had not yet been assessed at all until now. Hence, we investigated the relationships between organic C functional groups obtained from NEXAFS band areas and values of the corresponding C functionalities obtained from ^{13}C NMR and Sr-FTIR-ATR spectroscopy. Despite wide range of origin and chemical heterogeneity of the humic fractions, the results of C (1s) NEXAFS spectra deconvolution procedure compared very well with the results of the ^{13}C NMR spectroscopy. We found positive correlation for aromatic-C (quinone and aromatic C vs. H- and C-substituted aryl-C, $r = 0.72$), phenolic-C (phenolic-C vs. methoxyl-C and O-substituted aryl-C, $r = 0.88$), aliphatic-C (aliphatic-C vs. alkyl-C, $r = 0.70$), carboxylic-C (carboxylic-C vs. carbonyl-C, $r = 0.65$) and O-alkyl-C ($r = 0.63$) band areas identified by the C (1s) NEXAFS and ^{13}C NMR spectroscopy, respectively. These values are lower than the values reported for aromatic-C ($r = 0.91$), phenolic-C ($r = 0.98$), aliphatic-C ($r = 0.99$) and carbonyl-C ($r = 0.95$) by Schäfer *et al*. (2003) for fulvic acid samples extracted from ground water, but compare very well with the values reported from humic fractions extracted from subhumid tropical highland forest soils by Solomon *et al*. (2005b). Similarly, we observed positive correlations between the organic C functional groups identified by C (1s) NEXAFS and Sr-FTIR-ATR spectroscopy ($r = 0.70$ for aromatic-C (i.e. quinone and aromatic C vs. aromatic-C); $r = 0.45$ for phenolic-C; $r = 0.42$ for aliphatic-C (i.e. aliphatic-C vs. asymmetric and symmetric aliphatic-C (CH_3 and CH_2) stretching and aliphatic (C–H) deformation of CH_2 or CH_3 groups); $r = 0.67$ for carboxylic-C and $r = 0.62$ for O-alkyl-C (i.e. O-alkyl-C vs. polysaccharide-C), respectively. Our findings show that the characteristic band positions of NEXAFS spectra were sufficiently separated to allow discrimination of organic C functional groups using the deconvolution method. Therefore, it is possible to suggest that C K-edge NEXAFS spectroscopy is a useful technique not only for detecting C functionalities originating from heterogeneous organic matter sources but also to follow qualitatively and semiquantitatively the long-term molecular level structural dynamics of SOC following net C loss from soils due to anthropogenic perturbations. The results of our investigation provide clear evidence that the different spectroscopic techniques used in the present investigation have variable degrees of sensitivity for the different organic C functionalities. Although the general trends and patterns were similar, these techniques detect the impact of human-induced changes in SOC species to a different extent. Hence, it is imperative to use a suite of complimentary spectroscopic techniques in an integrated manner to accurately fingerprint the structural composition of SOC and to critically assess the long-term impacts of human-induced land-use and land-cover changes on the speciation and structural chemistry SOC to provide process-oriented data for global C and ecosystem models.

Conclusions

Our study clearly demonstrated that human-induced long-term land-use and land-cover changes clearly perturbed the soil ecosystem and led to an exponential depletion of up to 85% of the original organic C and N from the surface soils, thereby reducing the soil quality and the potentials of these native tropical and subtropical highland ecosystems to serve as C sinks. A significant proportion of total depletion occurred during the first 4 years of conversion indicating that these forest- and grassland-derived soils contain large amounts of labile SOM, potentially very vulnerable to degradation upon human-induced land-use and land-cover changes. We observed variable response to human intervention in the ecosystems investigated, whereby transitions from natural forest to agriculture lost higher proportions of SOC and N concentrations compared to conversions from native grasslands to arable cropping. The magnitude of changes observed from these tropical and subtropical highland soils were

much larger than the values reported in other temperate and tropical soils.

Using elemental analysis and integrated spectroscopic techniques (^{13}C NMR, Sr-FTIR-ATR and C (1s) NEXAFS spectroscopy), we were able to effectively fingerprint the structural composition of SOC in the humic fractions and provided unequivocal evidence that anthropogenic perturbations changed not only the sink capacity of soils through net C flux, but also considerably altered the inherent structural composition of the remaining SOC. These molecular level compositional changes were manifested by: (i) the continued degradation and loss of O-alkyl and acetal-C structures such as those found in carbohydrates and holocellulose (cellulose and hemicellulose), some labile aliphatic functionalities and side-chain oxidations of phenylpropane unit of lignin structures, and (ii) by continued aromatization and aliphatizaton of the humic fractions possibly through selective accumulation of a wide variety of H- and C-substituted aryl-C structures and recalcitrant aliphatic components such as (poly)-methylene units, respectively, following enhanced decomposition process. These changes appeared as early as the fourth year after anthropogenic interventions, and their intensity for the most part continued to increase with duration of cultivation until a new quasi-equilibrium was approached after 20 years. However, subtle but persistent changes in SOC functional group chemistry of the resultant SOM continued long after a steady state had been approached. These changes in the inherent molecular structures of the SOM may exert considerable influence on biogeochemical cycling of C and bioavailability of essential nutrients present in association with SOM as part of complex organic biomolecules, and may significantly affect the sustainability of agriculture, as well as potentials of the soils to sequester C in these tropical and subtropical agroecosystems. Our investigation also clearly highlighted the potentials and shortcomings of the state-of the art spectroscopic analytical tools employed to investigate the functional group level changes in SOC, and stressed the importance of method integration to critically assess the impacts of human-induced land-use and land-cover changes on speciation and structural composition of SOC to provide process-oriented data for global C and ecosystem models.

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