

Carbon K-Edge NEXAFS and FTIR-ATR Spectroscopic Investigation of Organic Carbon Speciation in Soils

Dawit Solomon,* Johannes Lehmann, James Kinyangi, Biqing Liang, and Thorsten Schäfer

ABSTRACT

Soil organic matter (SOM) is a fundamental component of soil and the global C cycle. We used C (1s) near-edge x-ray absorption fine structure (NEXAFS) and synchrotron-based Fourier transform infrared-attenuated total reflectance (FTIR-ATR) spectroscopy to speciate C and investigate the influence of land use on the composition of SOM in the humic substances extracted from clay and silt fractions. Soil samples were collected from natural forest, tea and *Cupressus* plantations and cultivated fields in Ethiopia. Carbon K-edge spectra revealed multiple C (1s) electron transitions in the fine structure of C NEXAFS region (284–290 eV) indicating the presence of aromatic-C, phenolic-C, aliphatic-C, carboxylic-C, and O-alkyl-C in the humic substances. It also exhibited good selectivity, where specific energy regions correspond to C in discrete functional groups. However, regions of slight overlap between 1s-3p/ σ^* transition of aliphatic-C and 1s- π^* transition of carboxylic-C may not be excluded. Fourier transform infrared-attenuated total reflectance spectroscopy showed larger proportions of aromatic-C (25.5%, 21.9%) and asymmetric and symmetric aliphatic-C (19.7%, 15.2%) groups in the silt than in clay, respectively. However, smaller proportion of polysaccharides (19.3%, 11.5%) was obtained from the silt compared with clay. The proportions of phenols (20.7%, 20.4%), aliphatic deformation of CH₂ or CH₃ (13.1%, 14.5%), and carboxylic (9.8%, 8.3%) groups were of similar magnitude in both fractions. The proportion of polysaccharides decreased in the order: natural forests > plantations > cultivated fields, while recalcitrant aromatic-C increased in the order: natural forest < plantation < cultivation. Therefore, C (1s) NEXAFS and synchrotron-based FTIR-ATR spectroscopy are powerful, nondestructive techniques that can potentially be used not only to identify and fingerprint complex structural characteristics of organic C macromolecules but also to investigate the impact of long-term anthropogenic management on the composition and biogeochemical cycling of organic C in terrestrial ecosystems.

THE INTERGOVERNMENTAL PANEL ON Climate Change (IPCC) recently confirmed that anthropogenic greenhouse effect is a reality (IPCC, 2001). Analysis of land-use changes through forest clearing to agricultural land has shown that global terrestrial ecosystems are net sources of C emission (1.7 ± 0.8 Pg C yr⁻¹) to the atmosphere (IPCC, 2001). Soils contain the principal organic C pool (1220–1550 Pg) of the terrestrial C reserves (2300 Pg) (Lal, 2001) and its transformation is an important element in the global C cycle. Land-use changes affect not only the amount but also the composition of SOM through their influence on the decomposition and humi-

fication processes (Solomon et al., 2002). Soil organic matter is a complex material consisting of plant biopolymers (e.g., polysaccharides, lignin, proteins, and cuticular materials), animal and microbial residues and humic substances (Chefetz et al., 2002). The amount, chemical composition, and polyelectrolytic characteristics of the resulting organic C, however, may vary considerably depending on the climate, origin, and age of the organic material (Chen et al., 2002). It is these changes and heterogeneities that have made the structural and functional characterization of soil organic C extremely challenging.

Several approaches using modern and fairly effective analytical techniques are available for the characterization of organic matter and humic substances associated with bulk soils and size separates. These techniques involve the use of (i) chemolytic (Kögel-Knabner, 1995; Zhang and Amelung, 1996; Solomon et al., 2002), (ii) pyrolysis (Saiz-Jimenez, 1994; Leinweber and Schulten, 1998), (iii) thermochemolysis (del Rio et al., 1998; Chefetz et al., 2002), (iv) compound-specific stable isotope and radiocarbon analysis (Eglinton et al., 1996; Macko et al., 1998), and (v) spectroscopic techniques (Gressel et al., 1995; Kögel-Knabner, 1997; Solomon et al., 2002; Pérez et al., 2004).

Although significant progress has been made in SOM investigations using these analytical techniques, advances in SOM characterization could further benefit from the progress made in nondestructive microscopic and microscale x-ray spectroscopy techniques such as scanning electron microscopy (SEM), energy dispersive x-ray (EDX) analysis, and x-ray photoelectron spectroscopy (XPS) (McKeague and Wang, 1980; McHardy and Robertson, 1983; Yuan et al., 1998) to get information about the chemical composition, microheterogeneity, and physical allocation of organic and mineral materials in soils (Kögel-Knabner, 2000; Scheinost et al., 2001). A more recent addition to the ensemble of microscale physical and chemical characterization techniques is synchrotron-based scanning transmission x-ray microscopy (STXM), a powerful method created through advances in x-ray microfocusing techniques and access to a high flux source of soft x-ray photons generated by synchrotron light sources. Synchrotron radiation is a light emitted by highly relativistic electrons as they transit the magnetic fields used to guide them along a closed orbit (Lobo et al., 2002). The emitted radiation extends

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Abbreviations: Ac, acetate; CEC, cation exchange capacity; FTIR-ATR, Fourier transform infrared spectroscopy-attenuated total reflectance; G, Gaussian peaks; HUMO, highest unoccupied molecular orbital; IPCC, Intergovernmental Panel on Climate Change; LUMO, lowest unoccupied molecular orbital; NEXAFS, near edge x-ray absorption fine structure; NMR, nuclear magnetic resonance; SOM, soil organic matter; STXM, scanning transmission x-ray microscopy.

from microwaves to the hard x-ray region. By connecting a tunable monochromator to the STMX, NEXAFS spectra close to the C K-edge, at the first 30 eV above the actual electron binding energy for C (284 eV) (Stöhr, 1992; Gutiérrez-Sosa et al., 1999; Rothe et al., 2000) can be collected. This region usually shows the largest variation in the x-ray absorption coefficient and is often dominated by intense narrow resonances (Stöhr, 1992). Carbon NEXAFS features arise from electronic transitions of an inner-shell electron to unfilled molecular orbitals or conduction bands. As the incident photon energy increases throughout an absorption edge, first structures associated with excitation to the lowest unoccupied molecular orbital (LUMO), which are π^* antibonding orbitals and mixed Rydberg/valence states for unsaturated molecules (double or triple covalent bonds) will appear. As the incident photon energy increases, the ionization threshold will be exceeded whereby the core electron may be completely removed from the influence of the core hole; that is, the electron will be promoted to continuum. Above the ionization threshold of C (approximately 290 eV) but still within the NEXAFS region, structures associated with higher energy unoccupied molecular orbitals (HUMO), typically of σ^* character associated with saturated (single) covalent bonds, and direct inner-shell ionization are anticipated (Stöhr, 1992; Cody and Botto, 1995; Scheinost et al., 2001). The unoccupied electronic structure and thus the inner-shell excited states are determined by the geometric and electronic (bonding) structure of the sample. These features make NEXAFS element specific and also very sensitive to the bonding environment thus making it a powerful tool to identify and fingerprint the chemical structure of organic C from the fine scale details of the absorption spectrum that occur at its edge. Carbon (1s) NEXAFS has been effectively employed in the past to study coal (Cody and Botto, 1995; Cody et al., 1995), humic substances (Rothe et al., 2000; Schmidt et al., 2000a) and biopolymers like amino acids and peptides (Boese et al., 1997). In these studies, the functional group chemistry of samples with unknown composition has been inferred from reference spectra in a qualitative way by comparing band height and shapes. More recently, however, Scheinost et al. (2001), Jokic et al. (2003), and Schäfer et al. (2003) have employed a semi-quantitative approach to interpret NEXAFS spectra in non-living organic matter in soils.

Numerous studies conducted using conventional source of infrared light in the past indicated that FTIR spectroscopy could provide an insight into structural characteristics of complex organic macromolecules such as humic substances (Schnitzer and Khan, 1972; Stevenson and Goh, 1971; Inbar et al., 1989; Haberhauer et al., 1998). The FTIR spectra of such macromolecules contain a variety of bands that are diagnostic and could serve as a valuable tool to characterize the principal classes of chemical groups of which SOM is comprised. In the present investigation, we employed synchrotron-based FTIR-ATR technique. Combining FTIR with synchrotron infrared light, which is up to 1000 times brighter than a conventional infrared source, may help us to

study the chemistry of samples that are smaller and/or more dilute in concentration in a nondestructive manner (Martin and McKinney, 1998). The use of ATR may provide an extra advantage by eliminating some of the problems associated with transmission infrared spectroscopy such as path length, concentration, and interference of water bands in the humic substance spectra due to the hygroscopic nature of KBr used for the preparation of conventional FTIR pellets (Tanaka et al., 2001).

The objectives of the present study were, therefore, (i) to assess the potentials of C (1s) NEXAFS and synchrotron-based FTIR-ATR spectroscopy to qualitatively and quantitatively characterize the functional groups of organic C and their distribution in organo-mineral particles of soils and (ii) to investigate the usefulness of these techniques to evaluate the impact of deforestation and land-use changes on the composition of organic C in size separates. The results from C (1s) NEXAFS and FTIR-ATR spectroscopy were related to the organic C functional groups determined using ^{13}C nuclear magnetic resonance (NMR) spectroscopy (Solomon et al., 2002).

MATERIALS AND METHODS

Site Description and Sample Preparation

The present study was conducted on soil samples collected from the southwestern highlands (Wushwush) and the southeastern Rift Valley escarpment (Munesa) of Ethiopia. Wushwush is located at 7°19' N lat. and 36°07' E long. The altitude of the area is 1900 m above sea level. Mean annual temperature is 18°C with an average annual precipitation of 1800 mm. The soils of the area have clayey texture, dark reddish brown color, and are classified as Plinthaquic Paleudalfs (Soil Survey Staff, 1999). The Munesa site is located at 7°35' N lat. and 38°45' E long. Mean annual temperature is 19°C with annual precipitation of 1250 mm. The soils at Munesa are well-drained, clayey textured and very dark reddish brown in color. They are classified as Typic Palehumults (Soil Survey Staff, 1999).

The land-use practices studied at the Wushwush site include natural forest, tea plantation (*Camellia sinensis* O. Kuntz) (35 yr old) and fields cultivated for 25 yr, while at the Munesa site *Podocarpus* dominated natural forest, *Cupressus* plantation (*Cupressus lusitanica* Mill.) (25 yr old) and fields cultivated for 30 yr were investigated. In the cultivated fields of both sites, maize (*Zea mays* L.) was grown without fertilizer inputs. However, during the intermittent dry periods, sorghum (*Sorghum bicolor* L. Moench) was grown at Munesa. The plowing depth both at the Wushwush and Munesa sites varies from 10 to 12 cm. Crop residues that remain on the fields are normally collected and used as animal feed at both sites. Detailed descriptions of the vegetation and other site characteristics of the study area were provided in Solomon et al. (2002). We selected three representative fields from each land-use system and collected nine subsamples in a radial sampling scheme (Wilding, 1985) using a core sampler (200 cm³ core volume) from the upper 10 cm of each field. A composite sample was then prepared from the subsamples. The samples were then air-dried and sieved (<2 mm) before fractionation and chemical analysis.

Physical Fractionation of Particle-Size Separates

After removing visible root remnants, 30 g of soil was ultrasonically treated with an energy input of 60 J mL⁻¹ using a probe type sonicator (Branson Sonifier W-450, Schwäbisch Gmünd, Germany) in a soil/water ratio of 1:5 (w/v). The coarse sand-size separates (250–2000 µm) were isolated by wet sieving. To completely disperse the remaining material in the <250-µm suspension, ultrasound was again applied with an energy input of 440 J mL⁻¹ in a soil/water ratio of 1:10 (w/v). The clay-size separates (<2 µm) were separated from the silt (2–20 µm) and fine sand (20–250 µm) size separates by repeated centrifugation. The silt size separates were separated from the fine sand-size separates by wet sieving. Coarse and fine sand separates were combined and finally all size separates were dried at 40°C before grinding them for chemical analysis. The detailed procedure of isolation of the different size separates is described elsewhere (Solomon et al., 2002).

Chemical Analysis

Carbon and N contents of the clay and silt fractions were analyzed by dry combustion using a C/H/N/S-analyzer (Elementar Vario EL; Elementar Analysensysteme GmbH, Hanau, Germany). The pH-KCl was determined in 1:2.5 (w/v) suspensions. Cation exchange capacity (CEC) was determined with 1 M NH₄OAc (ammonium acetate) (pH = 7.0) according to Avery and Bascomb (1974). Dithionite-citrate-bicarbonate-extractable Al and Fe (Al_d, Fe_d) as well as oxalate-extractable Al and Fe (Al_o, Fe_o) were determined using atomic absorption spectrometer (Varian AAS-400; Varian Techtron, Victoria, Australia) according to Mehra and Jackson (1960) and Blume and Schwertmann (1969). Selected soil physical and chemical characteristics are shown in Table 1.

Humic Substance Extraction

The extraction procedure of humic substances for NEXAFS and FTIR-ATR spectroscopy followed the outline in Solomon et al. (2003). The humic substances from the clay and silt fractions were extracted three times with a 0.1 M NaOH and 0.4 M NaF mixture at 1:5 (w/v) ratio and the combined extracts were filtered through a 0.2-µm membrane filter (Gelman Supor; Pall Gelman Laboratory, Ann Arbor, MI) under pressure. This procedure was repeated using the same membrane filter, where the partial plugging of the filter helps remove fine clay. Finally the extracts were dialyzed (Spectra/Por Membrane, MWCO, 12 000–14 000 Da; Spectrum Laboratories, Gardena, CA) and freeze-dried. The NaOH-NaF solution extracted from 52 to 64% of the total SOM from the particle-size separates of the soils under investigation.

Near Edge X-Ray Absorption Fine Structure Spectroscopy

Characterization of the humic substances extracted from the clay and the silt size separates using C (1s) NEXAFS was done at the X-1A1 beamline of the National Synchrotron Light Source (NSLS), Brookhaven National Laboratory using the STXM endstation. The essential components of the STXM 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 10⁶ 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 (Neuhäusler et al., 1999; Scheinost et al., 2001). The beamline slit width was set up at 40 µm for the entrance slit, 25 µm for the vertical exit slit and 25 µm for the horizontal exit slit. The monochromator was calibrated using the absorption band of CO₂.

Films of humic substance samples were prepared from the aqueous suspension obtained by dispersing them in Millipore water using ultrasound bath for sample uniformity. We transferred 3-µL droplets of the aqueous suspension to 100-nm thick Si₃N₄ windows (Silson Ltd, Northampton, England) and dried the suspension at 35°C. After high-resolution micrographs of the samples were taken using the STXM to locate the area of uniform thickness, the illuminated spot on the samples were then increased to 10 µm by defocusing the zone-plate. Spectra from the samples (I) were collected from three different spots through the films and SiN₃ windows by moving the grating from 280 to 310 eV on a single spot with 120 ms dwell times in steps of 0.1 eV. Before each sample scan, background spectra (I₀) were collected in triplicates (as mentioned above) through the sample free region of the same Si₃N₄ windows and averaged.

The spectra were deconvoluted using arctangent function (AT) for the ionization step at 290 eV 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 284.3 (G1), 285.0 (G2), 286.5 (G3), 287.3 (G4), 288.4 (G5), and 289.3 (G6) eV and two second higher (1s-2π*) transitions at (289.1 and 289.7 eV, data not shown) were resolved (Cody et al., 1998; Scheinost et al., 2001; Schäfer et al., 2003). Moreover, two Gaussian peaks for σ* transitions (σ¹ and σ²) were simulated with FWHM of <1 and <2 eV using WinXAS version 3.0 for windows (WinXAS Software, Hamburg, Germany). The spectra were pre-edge normalized using WinXAS version 3.1 to

Table 1. Some physical and chemical properties of surface soils (0 to 10 cm) collected from the sub-humid Ethiopian highlands.

Land use	Sand	Silt	Clay	pH	SOC†	N	C/N	CEC	Fe _d ‡	Fe _o §	Al _d ‡	Al _o §
	g kg ⁻¹			KCl	- g kg ⁻¹ soil -		cmol kg ⁻¹ soil	g kg ⁻¹				
Wushwush												
Natural forest	103	327	570	5.5	82	7.7	10.8	37.0	6.7	0.60	0.96	0.25
Tea plantation	102	330	568	4.5	42	4.0	10.4	24.7	6.5	0.70	0.97	0.32
Cultivation	70	337	593	4.7	38	3.7	10.2	26.4	5.8	0.83	0.77	0.30
Munesa												
Natural forest	130	347	523	6.8	98	7.9	12.6	52.2	2.5	0.30	0.38	0.15
Cupressus plantation	231	326	443	6.6	71	7.1	10.0	40.4	2.5	0.36	0.32	0.18
Cultivation	229	285	486	4.5	38	3.3	11.8	26.5	3.4	0.71	0.40	0.21

† SOC, soil organic carbon.

‡ Fe_d and Al_d, dithionite-citrate-bicarbonate extractable Fe and Al.

§ Fe_o and Al_o, oxalate extractable Fe and Al.

avoid spectral dependence on the total C content; therefore, spectral properties are indicative of changes in C chemistry (Jablonski et al., 2003). Since the fine structure in the C NEXAFS region above 290 eV transitions tends to be very broad and overlap (Cody et al., 1998; Schäfer et al., 2003), only main $1s-\pi^*$ transitions were used for subsequent quantification and interpretation of the C (1s) NEXAFS results in the present investigation.

Fourier Transform Infrared Spectroscopy

The synchrotron-based FTIR-ATR experiment was performed on the 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 32x transmission/reflection and FTIR step-scan spectrophotometer (Nicolet Magna 860, Thermo Nicolet Corp., Madison, WI) with a KBr beam splitter and mercury-cadmium-telluride (MCT) detector with 500- to 7000- cm^{-1} frequency range and 1.0 cm^{-1} spectral resolution.

Films of humic substance samples extracted from the clay and silt separates were prepared from the aqueous suspension obtained in similar manner as the ones for NEXAFS spectroscopy. We transferred 10- μL droplets of the aqueous suspension on infrared (IR) glass slides (Silon Ltd, Northampton, England) and the suspension was dried at 35°C in a vacuum oven. Spectra from the samples were recorded for IR measurements with a $10 \times 10 \mu\text{m}^2$ aperture size from 4000 to 650 cm^{-1} with a resolution of 4 cm^{-1} . Each spectrum was composed of 256 scans co-added before Fourier transform processing. After a background of the IR glass was subtracted, we used OMNIC version 6.1 for windows (Thermo Nicolet Corp. Madison, WI) on the reduced portions of the spectra (4000–800 cm^{-1}) to automatically correct the baseline, normalize, identify the peaks, and calculate the intensities of the spectra.

Nuclear Magnetic Resonance Spectroscopy

Liquid-state ^{13}C NMR spectra from the humic substances were recorded on a Bruker Avance DRX 500 NMR spectrometer (Bruker BioSpin GmbH, Rheinstetten, Germany). About 150 mg of the freeze-dried material was dissolved in 3 mL of 0.5 M sodium deuteroxide (NaOD) in a 10-mm NMR tube. Conditions for ^{13}C NMR were: spectrometer frequency, 125.8 MHz; inverse-gated decoupling; acquisition time, 0.33 s; delay time, 1.67 s; line-broadening factor, 100 Hz. Chemical shifts were recorded in ppm relative to the resonance of an external standard of TSP [3-(trimethylsilyl) propionic acid]. The signal areas were calculated by electronic integration (Solomon et al., 2002).

Statistics

Correlation coefficients showing the relationship between the organic C functional groups of humic substances identified by C (1s) NEXAFS and ^{13}C NMR chemical shifts were run using the software package SIGMA Plot Version 6.10 for windows (SPSS Inc., Chicago, IL).

RESULTS AND DISCUSSION

Speciation of Organic C Using C K-Edge NEXAFS Spectroscopy

The deconvoluted typical C (1s) NEXAFS spectra showing the main $1s-\pi^*$ transitions, the two σ^* transitions and the arctangent step function for humic substances extracted from the clay (<2 μm) fraction is

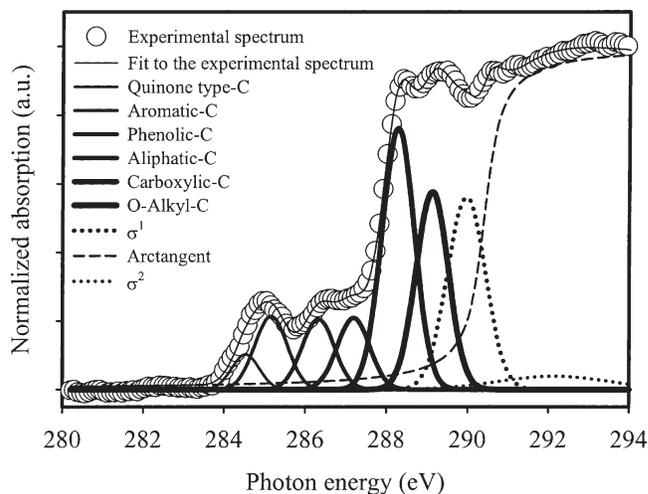


Fig. 1. Typical C (1s) NEXAFS spectra deconvolution showing the main $1s-\pi^*$ transitions, the two σ^* transitions and the arctangent step function (from left to right) from humic substances extracted from clay fraction (<2 μm) of a soil from Wushwush, Ethiopia. $1s-2\pi^*$ -transitions were not shown.

shown in Fig. 1. The spectra revealed multiple peaks in the fine structure of the C NEXAFS region (284–290 eV) indicating the presence of various C functional groups in the humic substances extracted from size separates of the soils under investigation. According to Fig. 1, the C $1s-\pi^*$ transition near 284.3 eV (G1) correspond to quinone type-C such as benzoquinone and to protonated and alkylated aromatic-C (Cody et al., 1998; Schäfer et al., 2003). The resonance near 285.0 eV (G2) represents molecular orbital of aromatic-C (protonated and alkylated to carbonyl-substituted aromatic-C) and possibly olefinic-C (Cody et al., 1998; Boyce et al., 2002; Jokic et al., 2003; Schäfer et al., 2003). The sum of these two organic C functional groups was used in subsequent discussions throughout this paper to represent aromatic-C. The absorption band near 286.5 eV (G3) is associated with phenolic-C including O-substituted aryl-C, which is indicative of lignin (Scheinost et al., 2001; Boyce et al., 2002; Schäfer et al., 2003). The $1s-3p/\sigma^*$ transition near 287.3 eV (G4) was due to aliphatic-C, whereas the strong absorption band near 288.4 eV (G5) was assigned to a $1s-\pi^*$ transition of carboxylic-C (Cody et al., 1998; Boyce et al., 2002; Jokic et al., 2003; Schäfer et al., 2003). This is in accordance with the fact that C is electron deficient in the C=O bonding. Consequently, due to the electron withdrawing nature of O in the C=O bonding, the corresponding 1s transition appears at energies approximately 3 eV above the C=C transition (Cody et al., 1998; Rothe et al., 2000). The sharp absorption band near 289.3 eV (G6) corresponds to the $1s-\pi^*$ of O-alkyl-C group representing mainly polysaccharides as well as smaller proportions of alcohol and ether C (Boyce et al., 2002; Scheinost et al., 2001). These results indicate that for the most part, C K-edge NEXAFS spectra obtained from the humic substances exhibited good selectivity, where specific energy regions tend to correspond to the C in discrete organic functional groups obtained from literature (Cody et al., 1998; Scheinost et al., 2001; Schäfer et al., 2003). However, regions of

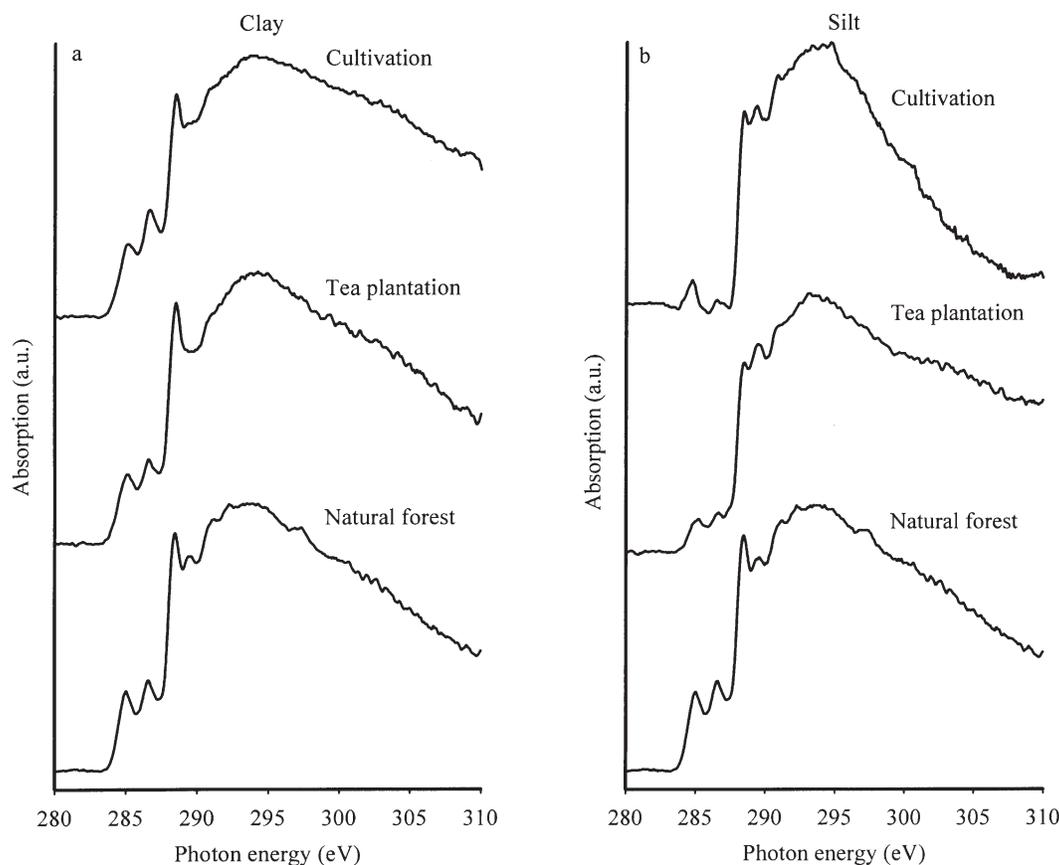


Fig. 2. Carbon (1s) NEXAFS spectra for humic substances extracted from the clay (<2 μm) and silt (2–20 μm) fractions of soils from Wushush, Ethiopia.

slight overlap between the bands associated especially with $1s\text{-}3p/\sigma^*$ transitions of aliphatic-C near 287.3 eV and $1s\text{-}\pi^*$ transitions carboxylic-C near 288.4 eV may not be excluded (Fig. 1). The existence of regions of overlap in C K-edge NEXAFS near 287 and 288 eV have been also reported in the past by Cody et al. (1998).

The C (1s) NEXAFS spectra and the relative proportions of different C functional groups in the humic substances extracted from clay and silt fractions of the Wushush and Munesa sites are shown in Fig. 2 and 3 and in Table 2 and 3, respectively. The C (1s) NEXAFS spectra of the humic substances extracted from clay fraction (Fig. 2a and Fig. 3a) indicate that this size separate was dominated by strong C $1s\text{-}\pi^*$ transition of carboxylic-C. This organic C functional group represented on average about 36.5% of the total organic C identified by C K-edge NEXAFS spectroscopy. The C $1s\text{-}\pi^*$ transition of O-alkyl-C is the second dominant organic C moiety accounting on average for 23.8% of the total organic C, while almost similar proportions of phenolic-C (14.6%) and aromatic-C (14.1%) were found in the clay fraction. The C $1s\text{-}3p/\sigma^*$ transition of aliphatic-C represented on average only 11.0% of the total organic C isolated by C NEXAFS spectroscopy from humic substances extracted from clay fraction of the soils under investigation (Table 2 and 3). Deconvolution of spectra from the silt fraction shows that the C $1s\text{-}\pi^*$ transition of carboxylic-C comprises on average 41.0%

of the total organic C in the humic substances associated with silt fraction. The C $1s\text{-}\pi^*$ transition of O-alkyl-C located near 289.3 eV represented on average 36.8%, while the primary peaks around 284.3 and 285.0 eV, which correspond to excitations of aromatic-C comprised 11.0% of the organic C identified by C K-edge NEXAFS spectroscopy from the silt fraction. The phenolic-C and $1s\text{-}\pi^*$ transition of aliphatic-C accounted on average for 6.3 and 4.9% of the organic C, respectively (Table 2 and 3). The results from both size fractions thus indicate that carboxylic-C and O-alkyl-C groups were the dominant forms of organic C, followed by moderate amounts of aromatic-C and phenolic-C groups. The aliphatic-C forms contributed only to a small portion of the total SOM associated with humic substances extracted from both the clay and silt fractions. These results are in line with the C (1s) NEXAFS results of Rothe et al. (2000) and Schäfer et al. (2003), where large amounts of carboxyl-C and O-alkyl-C moieties were reported in humic substances. They are also partially consistent with the results of Jokic et al. (2003), where large proportions of O-alkyl-C and carboxylic-C followed by aromatic-C and alkyl-C have been observed by C K-edge studies from the surface layers (approximately 10 nm) of wetland soils using total electron yield (TEY) mode. Jokic et al. (2003), however, reported an increase in the proportion of aliphatic-C compared with both carboxylic-C and aromatic-C from the same soils

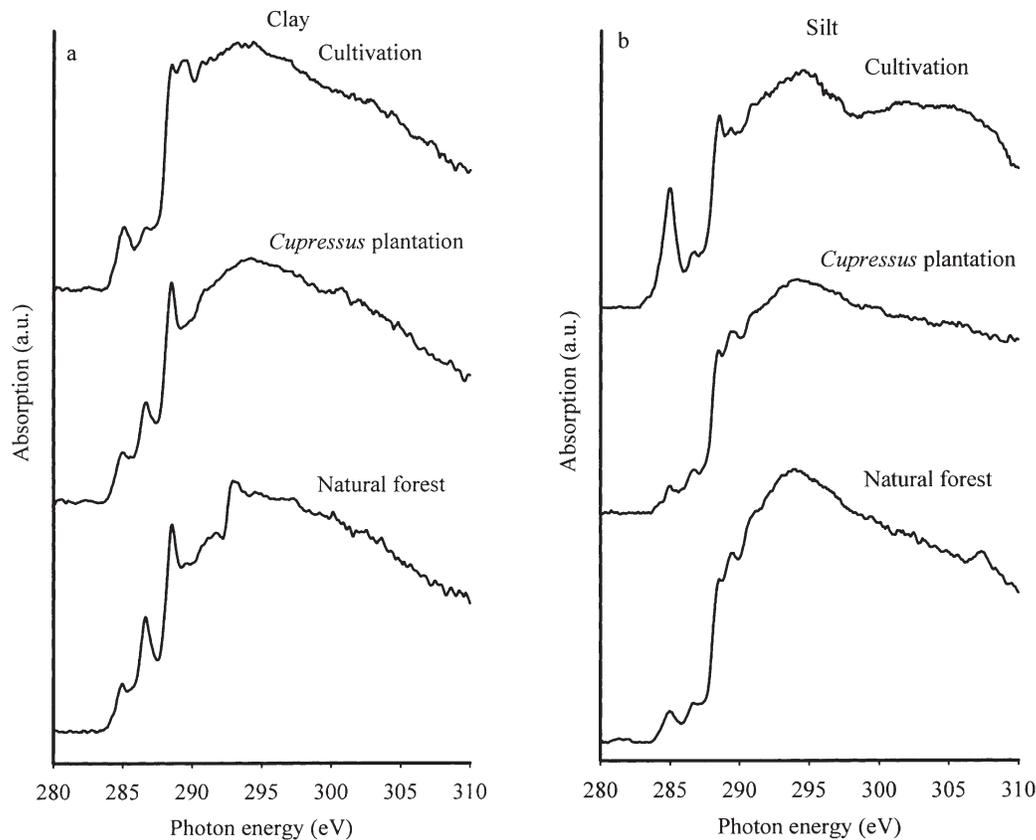


Fig. 3. Carbon (1s) NEXAFS spectra for humic substances extracted from the clay (<2 μm) and silt (2–20 μm) fractions of soils from Munesa, Ethiopia.

using fluorescence yield (FY) that probes deeper (approximately 100 nm) in to the sample. Examination of the NEXAFS spectral features revealed also small but consistent differences in the peak intensities of the organic C functional groups identified from the humic substances extracted from clay and silt fractions. The most prominent changes in the C K-edge NEXAFS spectra were indicated by the C 1s– π^* transition intensities between 284.3 and 288.4 eV (Fig. 2 and 3). The intensity of C 1s– π^* transitions of C functional groups between these absorption bands were generally larger and more well resolved in the clay fraction compared with the corresponding silt fraction. These features may reflect lower proportion of these organic C moieties in the SOM associated with the silt fraction.

Comparisons of the results from C K-edge NEXAFS

spectroscopy indicates that regardless of the large losses of SOC in the clay (from 22 to 44%) and silt (from 41 to 78%) following management changes (Solomon et al., 2002), the NEXAFS spectral features did not reveal clear and consistent differences in relative abundance of the various organic C functional forms in the humic substances extracted from the size separates of soils extracted from the contrasting land-use systems (Fig. 2 and 3 and Tables 2 and 3). These results are in accordance with previous studies (Skjemstad et al., 1986; Capriel et al., 1992; Guggenberger and Zech, 1999; Solomon et al., 2002) where, despite large losses in SOM contents following land-use changes, ^{13}C NMR spectroscopy failed to show significant differences in the structural make-up of SOM.

To evaluate the deconvolution procedure used for

Table 2. Relative proportions of organic C functional groups identified by C (1s) NEXAFS spectroscopy in the humic substances extracted from clay (<2 μm) and silt (2–20 μm) fractions of soils at Wushwush, Ethiopia.

Land use	Aromatic-C [†]	Phenolic-C	Aliphatic-C	Carboxylic-C	O-alkyl-C
%					
<u>Clay</u>					
Natural forest	16.0	12.8	10.7	36.3	24.2
Tea plantation	16.0	12.5	10.1	37.9	23.5
Cultivation	15.8	15.0	12.3	33.5	23.4
<u>Silt</u>					
Natural forest	8.1	5.2	5.9	46.5	34.3
Tea plantation	7.7	7.6	5.0	41.0	38.7
Cultivation	6.8	3.7	0.9	42.5	46.1

[†] Aromatic-C = quinone type-C and protonated and alkylated aromatic-C + aromatic-C and possibly olefinic C.

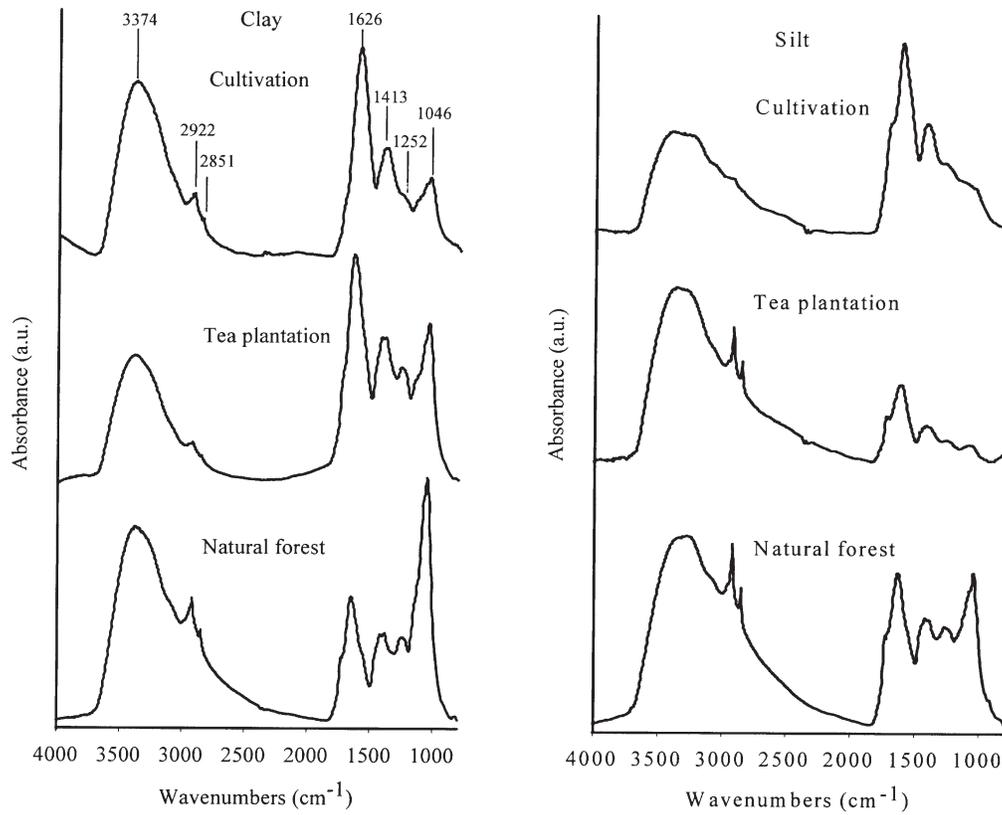


Fig. 4. Synchrotron-based FTIR-ATR spectra of humic substances extracted from the clay- and silt-size separates of soils from Wushush, Ethiopia.

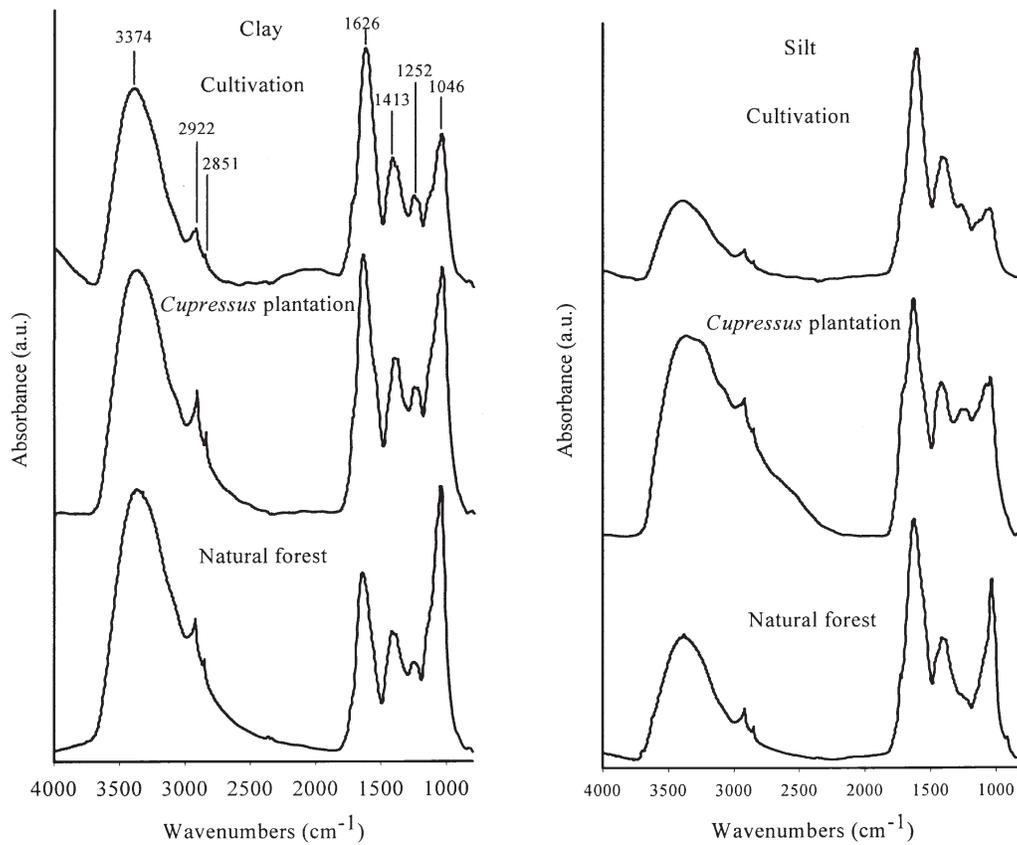


Fig. 5. Synchrotron-based FTIR-ATR spectra of humic substances extracted from the clay- and silt-size separates of soils from Munesa, Ethiopia.

Table 4. Relative proportions of the various organic C functional groups resolved from the peak intensities of the FTIR-ATR spectra of the humic substances extracted from clay (<2 μm) and silt (2–20 μm) fractions of soils at Wushwush, Ethiopia.

Land use	Phenol	Aliphatic [†]	Aromatic	Aliphatic [‡]	Carboxylic	Polysaccharide	Aromaticity [§]	Polysac-C/Arom-C [#]
	<u>Clay</u>							
Natural forest	20.5	22.6	13.1	9.2	8.9	25.7	0.13	1.96
Tea plantation	15.0	8.2	27.0	17.2	13.7	18.9	0.27	0.70
Cultivation	23.9	13.7	28.7	14.9	8.0	10.8	0.29	0.38
	<u>Silt</u>							
Natural forest	18.6	31.2	15.0	10.6	9.7	14.9	0.15	1.00
Tea plantation	31.0	41.7	13.7	6.5	4.0	3.1	0.14	0.22
Cultivation	21.4	4.9	40.0	23.2	6.3	4.2	0.40	0.10

[†] Aliphatic, weak stretch vibration of asymmetric and symmetric aliphatic (CH₃ and CH₂) groups.

[‡] Aliphatic, aliphatic (C-H) deformation of CH₂ or CH₃ groups.

[§] Aromaticity = (aromatic-C)/(Phenol-C + Aliphatic-C + Aromatic-C + Aliphatic-C + Aliphatic-C + Carboxylic-C + Polysaccharide-C).

[#] Polysac-C/Arom-C, (Polysaccharide-C/Aromatic-C).

by the FTIR-ATR spectroscopy in the humic substances extracted from the clay fraction, respectively (Table 4 and 5). Asymmetric and symmetric aliphatic-C groups accounted on average for 15.2% of SOM associated with the clay. The proportion of aliphatic-C deformation of CH₂ or CH₃ groups amounted for 13.1%, whereas C–O stretching and OH deformation of carboxylic (COOH) groups comprised only 9.8%. The spectral patterns and the proportions of the phenolic-C, aromatic-C, and polysaccharide-C moieties in the humic substance extracted from clay fraction were in reasonable agreement with the NEXAFS results from this size fraction. However, compared with the FTIR-ATR results, the proportion of carboxylic-C seems to be overestimated and the proportion of aliphatic-C underestimated by C K-edge spectroscopy. The FTIR-ATR spectra of the humic substances from silt fraction (Fig. 4b and 5b) exhibited distinct differences in the composition of organic C functional groups compared with the spectral pattern of the clay fraction. They contained larger proportions of aromatic-C (25.5%) as shown by a more pronounced absorption band at around 1626 cm⁻¹. This was also indicated by the higher ratio of aromaticity (aromatic-C/phenolic-C + aliphatic-C + aromatic-C + aliphatic-C + carboxylic-C + polysaccharide-C) in the silt (0.26) than in the clay (0.22) fraction (Table 4 and 5). In contrast to the clay, the silt fraction showed slightly larger proportions of asymmetric and symmetric aliphatic-C (CH₃ and CH₂) (19.7%) groups. However, both the peak intensities and the average proportions of the

C–O stretching vibrations of polysaccharides (11.5%) were much smaller in the humic substances extracted from silt than from clay-size fractions. This was further demonstrated by the lower average value for the ratio of polysaccharide-C/aromatic-C (polysac-C/arom-C) in the humic substances extracted from silt (0.51) fraction compared with the humic substances extracted from clay (1.02) fraction (Table 4 and 5). These results clearly indicate that the SOM associated with the silt fraction was more aromatic compared with the SOM associated with the clay fraction, while the humic substances extracted from the silt contain lower amount of carbohydrates than the ones extracted from the clay. They also supports our previous study on these soils using ¹³C NMR spectroscopy and wet-chemical analysis, where we found lower aromaticity and larger enrichment of carbohydrates (especially microbial-derived sugars such as galactose, mannose, fucose, and rhamnose) in SOM associated with the clay than with the silt fraction (Solomon et al., 2002). Loss of O-alkyl C structures and an increased accumulation of aromatic-C (probably from lignin and tannins) in silt compared with clay have been also observed by Schmidt et al. (2000b). The fact that potentially labile polysaccharides accumulate in the clay fraction could be attributed to the higher production of microbial metabolites and their intimate association and stabilization against mineralization in clay minerals (Oades et al., 1987; Guggenberger et al., 1994; Solomon et al., 2000). Schmidt and Kögel-Knabner (2002) suggested that sorptive organo-mineral interactions with

Table 5. Relative proportions of the various organic C functional groups resolved from the peak intensities of the FTIR-ATR spectra of the humic substances extracted from clay (<2 μm) and silt (2–20 μm) fractions of soils at Munesa, Ethiopia.

Land use	Phenol	Aliphatic [†]	Aromatic	Aliphatic [‡]	Carboxylic	Polysaccharide	Aromaticity [§]	Polysac-C/Arom-C [#]
	<u>Clay</u>							
Natural forest	22.9	19.6	15.6	10.5	7.9	23.5	0.16	1.50
Cupressus plantation	19.7	16.6	20.9	12.6	10.3	19.9	0.21	0.95
Cultivation	22.0	10.2	26.4	14.3	10.2	16.9	0.26	0.64
	<u>Silt</u>							
Natural forest	15.4	10.6	29.5	15.0	7.4	22.1	0.29	0.75
Cupressus plantation	17.9	21.9	21.1	13.7	11.3	14.1	0.21	0.67
Cultivation	18.1	8.0	33.8	18.2	11.2	10.7	0.34	0.32

[†] Aliphatic, weak stretch vibration of asymmetric and symmetric aliphatic (CH₃ and CH₂) groups.

[‡] Aliphatic, aliphatic (C-H) deformation of CH₂ or CH₃ groups.

[§] Aromaticity = (aromatic-C)/(Phenol-C + Aliphatic-C + Aromatic-C + Aliphatic-C + Aliphatic-C + Carboxylic-C + Polysaccharide-C).

[#] Polysac-C/Arom-C, (Polysaccharide-C/Aromatic-C).

clay size minerals with large surface areas could protect polysaccharides from degradation and may lead to their accumulation in clay fraction. The average proportions of H-bonded hydroxyl (O–H) groups of phenols (O–H) (20.4%), aliphatic (C–H) deformation of CH₂ or CH₃ (14.5%), and C–O stretching and O–H deformation of carboxylic (COOH) (8.3%) groups were of similar magnitude in the two size fractions.

The FTIR-ATR spectra (Fig. 4 and 5) and the relative proportions of the signal intensities (Table 4 and 5) also clearly demonstrated the long-term impact of land-use on the different organic C functional forms present in the size separates of these tropical soils. The most striking changes in the signal intensities of FTIR-ATR spectra of the humic substances extracts of particle-size fractions under different land-use systems appeared near 1046 and 1626 cm⁻¹ regions, which represent C–O stretching vibrations of polysaccharides and aromatic C (C=C), respectively (Fig. 4 and 5). The FTIR-ATR spectra of humic substances from the natural forests were distinguished from the plantations and cultivated soils by more intense C–O stretching vibrations of the polysaccharide region. According to Table 4 and 5, this signal intensity expressed as the relative proportions of C–O stretching vibrations of polysaccharides decreased in the order: natural forests > plantations > cultivated fields both in the clay and silt fractions at the Wushwush and Munesa sites. The decrease in signal intensities and relative proportions following land-use changes reflected an enrichment of carbohydrates in the humic substances extracted from the size separates under natural forests compared with the corresponding size separates under long-term plantation and continuous cultivation. The lower carbohydrate concentrations in the arable soils may be attributed to the lower biomass input coupled with the rapid depletion of polysaccharides due to accelerated mineralization of the original SOM following clear-cutting and long-term continuous cultivation under the subhumid tropical highland environment. These results are in agreement with our previous study on these soils using degradative wet-chemical analysis and ¹³C NMR spectroscopy, where we found lower concentrations of non-cellulosic carbohydrates in SOM associated with the size separates of the plantations and continuously cultivated soils compared with the corresponding size separates of the natural forests (Solomon et al., 2002). In contrast, the absorption band intensity and the relative proportion of aromatic-C (C=C) vibrations increased in the order: natural forest < plantation < cultivation at both sites, indicating that more recalcitrant aromatic forms are becoming the dominant forms of organic C functional groups in the humic substances extracted from the plantation and cultivated soils following land-use changes. The above results were also supported by the polysac-C/arom-C ratios and by the degree of aromaticity of the humic substances (Table 4 and 5). According to Table 4 and 5, the ratio of polysac-C/arom-C decreased considerably after clear-cutting and subsequent establishment of plantations or cultivated fields both in the humic substances extracted clay (from 1.96 and 1.50 to 0.38 and 0.64) and silt (from

1.00 and 0.75 to 0.10 and 0.32) fractions of the soils at the Wushwush and Munesa sites, respectively. However, the degree of aromaticity in the humic substances increased from 0.13 and 0.16 to 0.29 and 0.26 in the clay fraction and from 0.15 and 0.29 to 0.40 and 0.34 in silt fraction due to agricultural management at the two sites, respectively. Our results concur positively with the results of Golchin et al. (1995) and Baldock et al. (1997) who suggested that tillage of native soils can cause loss of more readily decomposable fractions, potentially leading to an increase in the proportions of the more recalcitrant aromatic C forms in the SOM at the advanced stage of decomposition. They also compared positively with the statistical analysis of published ¹³C NMR data by Mahieu et al. (1999), which showed that humic acids from cultivated soils have higher concentration of aromatic C than humic acids from non-cultivated soils.

The FTIR-ATR spectral features and the peak intensity values also indicated accumulation of some aliphatic structures in the humic substances extracted from the size separates of soils from the natural forests and plantations than the cultivated fields. This was shown by the intensities of the peaks produced by vibrations of asymmetric and symmetric aliphatic (CH₃ and CH₂) groups (Fig. 4 and 5 and Table 4 and 5). These findings are in general agreement with previous investigations on the fate of organic C moieties during composting of organic matter (Inbar et al., 1989) or humification of SOM in forest-derived soils (Kögel-Knabner, 1993; Haberhauer et al., 1998), where a decrease of aliphatic chains following decomposition of organic matter was observed. However, the decrease in the signal intensities of the asymmetric and symmetric aliphatic groups was not complemented by the trend of aliphatic (C–H) deformation of CH₂ or CH₃ groups, which showed a small but consistent increase in the peak intensities in the humic substances extracted from the cultivated soils. In comparison, the changes in the relative proportions of C–O stretching and OH deformation of carboxylic (COOH) groups and H-bonded hydroxyl (O–H) groups of phenols in the humic substance extracts following land-use changes were small and inconsistent at both sites.

Haberhauer et al. (2000) and Pérez et al. (2004) suggested that although FTIR spectroscopy can be used for identification of functional groups and determination of their relative distribution, it does not provide detailed information about chemical or structural changes in humic acids in studies involving short-term conversions of forest to cultivated land or pastures. On the contrary, the changes in ratios, proportions and peak intensities of the FTIR-ATR spectral patterns observed in the present investigation clearly indicate that easily degradable SOM constituents such as polysaccharide-C and some aliphatic-C moieties were oxidized due to accelerated mineralization following land clearing and long-term cultivation. The remaining organic matter in the humic substances extracted from the size separates of the agriculturally managed soils was dominated by recalcitrant aromatic-C structures, clearly indicating a change in the

composition and structural stability of the SOM following long-term land use changes. Hence, it is possible to suggest that synchrotron-based FTIR-ATR spectroscopy is a powerful, non-evasive technique that has potential to obtain detailed information on the complex structural characteristics of organic macromolecules, determine their relative distribution and effectively follow the changes brought by long-term land-use changes on the composition of novel SOM.

CONCLUSIONS

With the help of C (1s) NEXAFS spectroscopy, we were able to effectively identify and fingerprint the various functional groups representing molecular structure of organic C from the fine scale details of the absorption spectra that occur at the C K-edge in the humic substances extracted from the clay and silt fractions of soils. Besides, we were able to investigate the distribution and impact of land-use changes on the amount and composition of organic C moieties in humic substances extracted from these sizes separates qualitatively and semi-quantitatively.

The NEXAFS spectra showed that carboxylic-C and O-alkyl-C functional groups were the dominant forms of organic C, followed by moderate amounts of aromatic-C and phenolic-C groups. The aliphatic-C forms contributed only to a small portion of the total SOM associated with the humic substances extracted from the two size fractions. Carbon K-edge NEXAFS spectroscopy, for the most part exhibited good selectivity, where specific energy regions tended to correspond to the C in discrete organic functional groups. However, regions of slight overlap between the bands associated especially with $1s-3p/\sigma^*$ transition of aliphatic-C and $1s-\pi^*$ transition of carboxylic-C may not be excluded. The NEXAFS spectral features did not reveal clear and consistent differences in relative abundance of the various organic C functional groups in the humic substance extracts of the size fractions under the different land-use systems.

Synchrotron-based FTIR-ATR spectroscopy, however, clearly demonstrated the long-term impact of land-use changes on the amount, composition, and structural stability of the different organic C functional forms present in the humic substances extracted from the size separates. Easily degradable SOM constituents such as polysaccharide-C and some labile components of aliphatic-C moieties were more prominent in the humic substance extracted from the size fractions of native forest soils, while aromatic-C and some recalcitrant forms of aliphatic-C forms dominated the SOM associate with the humic substances extracted from the size separates of soils from the plantations and continuously cultivated fields.

Therefore, C K-edge NEXAFS and synchrotron-based FTIR-ATR spectroscopy are powerful, nondestructive techniques that can potentially be used not only to identify and fingerprint the complex structural characteristics of organic C macromolecules but also to determine the impact of long-term anthropogenic management on

the composition and biogeochemical cycling of organic C in terrestrial ecosystems.

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