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Changes of humic substances characteristics from forested to cultivated soils in Ethiopia

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Abstract

The dynamics of the stable fractions of soil organic matter (SOM), the humic substances, has been rarely studied in tropical soils. We evaluated extraction yields, elemental composition, isotopic $\delta^{13}\text{C}$ abundance and solid-state ^{13}C NMR spectra of humic fractions isolated from five forested and cultivated soils in Ethiopia in order to assess the influence of deforestation and subsequent cultivation on the composition of humic fractions. In all locations, humic (HA) and fulvic (FA) acids, humin (HU) and a humic hydrophobic fraction extracted with an acetone–HCl solution (HE) were decreased as a result of deforestation and cultivation, but the magnitude of decrease was site-specific. While the elemental composition of HA, FA and HU did not vary significantly with land use, that of the hydrophobic HE generally decreased with deforestation. Cultivation varied the $\delta^{13}\text{C}$ values of humic fractions with location and their high values suggested a history of C4 plants incorporation in soil. The ^{13}C NMR spectra of HA, FA and HU showed that hydrophobic components (HB) were generally lower than hydrophilic ones (HI), resulting in a degree of hydrophobicity (HB/HI) being < 1.00 . On average, cultivation decreased the HB/HI values of these humic fractions in all soils except for the Andisol. The HE material was much richer in hydrophobic groups showing a HB/HI ranging from 1.48 to 3.44 in the forested sites, but cultivation substantially reduced HB/HI ratios also for HE. Our data suggest that deforestation and progressive cultivation in tropical conditions decreased the content of soil humic substances and that the resulting humic pool was poorer in alkyl components and less hydrophobic than in forested conditions.

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Keywords: Tropical soils; Humic substances; Organic matter dynamics; Chemical indicators; CPMAS- ^{13}C NMR; Land use effects

1. Introduction

The study of SOM is important for its role as a source and reservoir of atmospheric CO_2 and

plant nutrients and a main factor linking the physical, chemical and biological properties of soils (Duxbury et al., 1989). Soil organic matter is composed of different compartments which differ from each other in biochemical composition, biological stability and carbon turnover rates (Paustian et al., 1992). The humified SOM (HA,

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FA and HU) represents the most microbially recalcitrant and thus stable reservoir of organic carbon (OC) in soil and an important component for the control of soil erosion by water and wind (Piccolo, 1996).

Most studies on SOM dynamics in the tropics have focused on its absolute amount without recourse to their chemical fractions (Scholes et al., 1997). Soil physical fractionation techniques have been used to evaluate the potential of sub-tropical soils to increase OC sequestration under no-till management (Bayer et al., 2001; Sà et al., 2001). Few studies carried out in Brazil (Andreux et al., 1994; Bravarda and Righi, 1991; Nascimento et al., 1992) and Venezuela (Paolini and Chitty, 1990; Rivero et al., 1998) have employed standard fractionation techniques of humic substances to assess the stability of the humic pool in tropical soils. Kunda et al. (1994) and Feller et al. (1991) have discussed the C and N contents of humic fractions of SOM in tropical Africa. Zech et al. (1997) reviewed several studies that have utilized solid-state NMR spectroscopy (CPMAS-¹³C NMR) to characterise the humification process of SOM in the tropics. However, no specific attempts have been made to monitor the chemical characteristics of humic substances and their modifications when forested sites are cleared for intensive cultivation, especially in the tropical highlands such as those found in Ethiopia. Yet SOM and HAs, in particular, play important roles in these soils where 1:1, low-activity clays, and low cation exchange capacity (CEC) are common. In these conditions, more than 70% of the CEC of such soils and, hence, of their chemical fertility is attributed to SOM (Asadu et al., 1997).

The objective of this study was to characterize the humified OM fractions from the highland soils of Ethiopia, representing a cool, tropical ecosystem. This study is an attempt to fill the gap in our knowledge of the dynamics of humic matter in African ecosystems especially when forested sites are turned into cultivation. A further understanding on the changes of the molecular composition of humified SOM may be the basis for the development of indicators predicting potential carbon storage and resistance to erosion in tropical soils.

2. Materials and methods

2.1. Soil sampling and preparation

This study was conducted with soil samples from five forested and cultivated locations in Ethiopia belonging to well-established experimental sites of the Institute of Agricultural Research (IAR) of Ethiopia. A low-input farming system involves clearing of forested sites for arable crop production without external inputs of fertilizer or organic residues. Cultivation consists of disking, ploughing and harrowing in Holeta, Ginchi and Jimma while essentially hand hoeing and planting without mechanical tillage were used in Awassa and Sirinka. Geographical information on the study sites are given elsewhere (Spaccini et al., 2001). Relevant properties of soil samples are reported in Table 1.

The experiment was set up as a Randomized Complete Block Design (RCBD) with three replicates of each of the forested and cultivated treatments. Each plot was 6 × 4 m in size and had been subjected to the above practices since 1990. From September to November 1995, three bulked topsoil (0–20 cm) samples, collected from each replicate of the treatments, were air-dried, sieved through a 4.75 mm sieve and shipped to Italy for fractionation of humic substances and their characterization.

The < 2.00 mm (fine earth) fractions were used to determine particle size distributions by the pipette method of Gee and Bauder (1986). The soil pH was measured in 1:2.5 soil/water ratio, OC by the wet oxidation method of Nelson and Sommers (1982), and NH₄OAc-exchangeable bases and CEC by the procedures described by Thomas (1982) and Rhoades (1982), respectively.

2.2. Extraction of humic substances

A series of humic fractions were isolated from soil samples as described elsewhere (Piccolo et al., 1998). The following fractions were obtained:

1. HA and FA were extracted under N₂ atmosphere with a combined 1 M NaOH and 1 M Na₄P₂O₇ solution (50:50, v/v).
2. HU was extracted from the soil residue remaining after the NaOH–Na₄P₂O₇ extraction of HA and FA.

Table 1
Sampling site, soil classification, vegetation type and some properties of the Ethiopian soils of this study

Location	Soil order	Vegetation	Texture ¹	Clay minerals ²	pH (H ₂ O)	OC (g kg ⁻¹)	CEC (cmol _c kg ⁻¹)
Awassa 06°58'N, 38°25'E	Andisol	Highland savannah	LS	Hy ^a , Mi ^b , Sm ^b	5.28 (0.19)	Forested 40.3 (5.5)	30.4 (1.1)
					5.45 (0.23)	Cultivated 16.6 (4.1)	22.6 (1.3)
Ghinchi 09°01'N, 38°20'E	Vertisol	Savannah	C	Sm ^a , Mi ^b , K ^c	5.74 (0.18)	Forested 32.8 (2.9)	54.4 (2.1)
					5.98 (0.23)	Cultivated 20.0 (5.1)	54.0 (1.3)
Holeta 09°03'N, 38°30'E	Vertisol	Savannah	C	Mi ^a , K ^b Hy ^b , Sm ^b	5.58 (0.22)	Forested 36.1 (4.5)	31.8 (2.7)
					4.88 (0.19)	Cultivated 16.0 (2.3)	29.6 (1.9)
Jimma 07°41'N, 36°50'E	Alfisol	Rainforest	C	Mi ^a , Hy ^a , K ^c	6.15 (0.23)	Forested 47.3 (4.9)	30.2 (1.5)
					4.94 (0.18)	Cultivated 20.2 (6.5)	26.2 (1.8)
Sirinka 11°62'N, 39°70'E	Vertisol	Guinea savannah	C	Sm ^a , Mi ^b , K ^c	6.02 (0.29)	Forested 27.2 (5.5)	56.0 (3.8)
					6.75 (0.24)	Cultivated 7.7 (1.4)	60.0 (2.9)

1. LS=loamy sand; C=clayey.

2. Mi=mica; Sm=smectite; K=kaolinite; Hy=halloysite; a=abundant; b=present; c=few.

The soil residue was first washed with distilled water until neutrality of washings and then shaken twice with a 2 M HF solution for 24 h. After centrifugation, the supernatant was discarded while the residue was washed to neutrality with distilled water and again extracted with a 1 M NaOH–Na₄P₂O₇ solution to obtain the HU fraction. This was then purified as for HA.

- The HE fraction was solubilized in a solution of acetone–0.6 M HCl (8:2, v/v). Briefly, soil samples were placed in stoppered polyethylene bottles with the extracting solution (1:5, w/v) and shaken for 24 h on a mechanical shaker. The mixture was centrifuged, and the supernatant containing the extract (HE) was filtered through a quartz filter. The soil residue was further extracted twice with the same extracting solution. The HE from subsequent extractions was combined, rotoevaporated and dialyzed against water to remove the solvent completely.

For the alkaline extractions, the suspensions holding HA and FA, once filtered through a glass wool, were combined and rapidly brought to pH 1 with 12 M HCl, and the HA was allowed to precipitate for 24 h at 20 °C. The precipitated HA was purified from inorganic impurities through repeated (three times) dissolution and re-precipitation in 0.5 M NaOH and 0.5 M HCl solutions, respectively. The soluble FA was purified by adsorption on an acid-conditioned non-ionic polymeric resin (XAD-8, Serva Chemicals) to remove the low-

molecular-size hydrophilic organic compounds. The FA were desorbed by alkaline solution, brought to pH 5, dialyzed against distilled water until chloride-free, freeze-dried and stored for further analysis. The HA, HU and HE samples were further purified by shaking in polyethylene bottles for 24 h with a solution of 0.062 M HCl and 0.114 M HF, dialyzed against water until free of Cl⁻, freeze-dried and stored for further analysis.

2.3. NMR spectroscopy

The ¹³C NMR spectra of humic extracts from the soils were recorded by the Cross Polarization Magic Angle Spinning (CPMAS) technique. The analyses were done with a Bruker AMX400 at 100.625 MHz using a rotating speed of 5000 ± 50 Hz, a contact time of 1 ms, a recycle time of 1 s and an acquisition time of 13 ms. All runs were made with Variable Contact Time (VCT) pulse sequence in order to find the Optimum Contact Time (OCT) for each sample and to minimize the error on the evaluation of the peak areas (Conte et al., 1997). The OCT ranged between 0.8 and 1.0 ms. The line broadening for Free Induction Decay (FID) transformation was fixed at 50 Hz. The following resonance intervals are generally attributed to different carbons (Wershaw, 1985): alkyl C: 0–50 ppm; C–O and C–N in ethers, esters, carbohydrates, amines: 50–85 ppm; C–O in carbohydrate (acetal) compounds: 85–105 ppm; aromatic C: 105–160 ppm; carbonyl-related C: 160–200 ppm. The areas relative to these resonance

intervals were used to evaluate the degree of hydrophobicity (Hydrophobic/Hydrophilic carbons, HB/HI) of the humic fractions:

$$HB/HI = [(0 - 50) + (105 - 160)] / [(50 - 105) + (160 - 200)].$$

All integral regions were also corrected for the areas of spinning side bands (SSB) when they appeared in the spectra. Each SSB area was subtracted from that of the region in which it appeared and added to the area of the centre band (Wilson, 1987; Piccolo and Conte, 2003).

2.4. Elemental composition and $\delta^{13}\text{C}$ content of humic fractions

The isotopic abundance ($\delta^{13}\text{C}$) in the isolated humic samples was measured by continuous-flow isotope ratio-mass spectrometry (Carlo Erba N Analyzer 1500 coupled with a Finnigan MAT 251). The results of the stable isotope $\delta^{13}\text{C}$ abundance are expressed, using the δ notation per mil (‰), as the deviation of the isotopic ratio of the sample from that of an arbitrary standard:

$$\delta^{13}\text{C}\text{‰} = [(R - \text{sample} - R - \text{standard}) / (R - \text{standard})] \times 1000,$$

where $R - \text{sample} = {}^{13}\text{C}/{}^{12}\text{C}$ of the sample, and $R - \text{standard} = {}^{13}\text{C}/{}^{12}\text{C}$ of the Pee Dee Belemnite (PDB) standard ($R - \text{PDB} = 0.0112372$).

The C, N and H contents in all humic fractions were calculated on ash-free bases from measurements obtained by a Fison-Interscience elemental analyser.

3. Results and discussion

3.1. Yields of humic fractions

The yields of extraction for HA, FA, HU and the humic fraction solubilized in the acetone–HCl solution (HE), obtained from the sites under study are shown in Table 2. In accordance with the loss of SOM (Table 1), a progressive decrease in these humic fractions was found for all soils as forests were converted to arable farming. The magnitude of

Table 2

Extraction yields (g kg^{-1} of soil) of HA, FA and HU and humic extracts (HE) solubilized with an acetone–HCl solutions from forested (F) and cultivated (C) sites in Ethiopia

Location	Sites	HA	FA	HU	HE
Awassa	F	15.7 ± 0.5	6.0 ± 0.3	2.9 ± 0.4	2.7 ± 0.6
	C	11.5 ± 0.7	6.4 ± 0.4	0.7 ± 0.2	1.7 ± 0.5
Ghinchi	F	13.3 ± 0.3	2.5 ± 0.4	0.6 ± 0.1	3.1 ± 0.2
	C	9.1 ± 0.8	1.1 ± 0.2	0.7 ± 0.1	0.7 ± 0.3
Holeta	F	7.7 ± 0.3	4.0 ± 0.8	1.3 ± 0.3	4.4 ± 0.5
	C	3.3 ± 0.1	2.2 ± 0.3	1.3 ± 0.5	2.3 ± 0.4
Jimma	F	12.5 ± 0.1	6.8 ± 0.8	4.2 ± 0.3	3.6 ± 0.7
	C	3.8 ± 0.8	0.8 ± 0.2	3.2 ± 0.5	0.4 ± 0.2
Sirinka	F	10.5 ± 0.9	1.3 ± 0.7	0.3 ± 0.8	2.4 ± 0.6
	C	2.3 ± 0.6	1.2 ± 0.6	0.3 ± 0.5	1.4 ± 0.5

decrease was, however, site-specific. More than 75% of diminution in HE occurred at Ghinchi and Jimma whereas the least occurred at Awassa. Jimma and Sirinka showed a large decrease in HA (69% and 78%, respectively) whereas Jimma recorded the largest reduction in FA (88%) and Awassa in HU (75%). From the magnitude of the changes it is evident that deforestation and subsequent cultivation generally decreased the relative content of HE and HA whereas that of FA and HU were less affected. Similar diminution in these humic fractions with cultivation have been reported in previous works (Mbagwu and Piccolo, 1998; Spaccini et al., 2000). Such decrease is commonly attributed to microbial oxidation of the organic materials previously protected in the soil aggregates which were destroyed by cultivation (Cambardella and Elliot, 1993; Piccolo, 1996). Our findings indicate that cultivation affected the studied soils in their content of HE and HA more than in other humic fractions.

3.2. Elemental content and $\delta^{13}\text{C}$ values of humic fractions

The C and N contents and the C/N ratios of HA, FA and HU fractions (Table 3) were within the ranges reported for other soils (Stevenson, 1994) and did not substantially change from forested to cultivated soils. The general constancy of C/N values for the different humic fractions suggests a resistance to microbial decomposition attributable to their recalcitrant nature deriving from either physical protection in micro-aggregates (Dutarte et al., 1993) or chemical protec-

Table 3
C and N contents (g kg⁻¹) and $\delta^{13}\text{C}$ (‰) values of humic fractions (HA, FA, HU) from forested (F) and cultivated (C) sites of Ethiopia

Location	Fraction	Site	C	N	C/N ratio	$\delta^{13}\text{C}$
Awassa	HA	F	566.9	32.7	17.3	-14.45
		C	584.0	33.6	17.4	-15.97
	FA	F	332.3	24.6	13.5	-14.28
		C	343.8	24.9	13.8	-18.47
	HU	F	509.9	37.0	13.8	-14.64
		C	487.9	34.6	14.3	-16.75
Ghinchi	HA	F	566.1	27.8	20.4	-14.61
		C	374.5	20.4	18.3	-15.63
	FA	F	390.0	32.7	11.9	-14.83
		C	274.5	15.2	18.0	-18.50
	HU	F	496.1	26.6	18.6	-14.43
		C	478.8	25.0	19.1	-16.52
Holeta	HA	F	548.3	31.4	17.5	-17.61
		C	510.3	34.0	15.0	-16.17
	FA	F	373.7	25.4	14.5	-17.29
		C	364.4	19.2	18.0	-15.33
	HU	F	483.3	24.8	19.9	-20.13
		C	454.8	23.7	19.2	-15.23
Jimma	HA	F	529.8	38.1	13.9	-19.48
		C	528.4	42.7	12.4	-17.26
	FA	F	394.4	28.3	13.9	-19.57
		C	383.2	29.0	13.2	-17.45
	HU	F	539.4	39.3	13.7	-19.54
		C	533.5	20.1	26.5	-14.40
Sirinka	HA	F	575.4	29.8	19.3	-13.98
		C	582.3	28.7	20.3	-14.45
	FA	F	305.8	27.1	11.3	-14.11
		C	219.2	13.5	16.2	-16.75
	HU	F	550.8	35.4	15.5	-14.04
		C	546.6	32.4	16.9	-15.03

tion by their hydrophobic composition (Spaccini et al., 2000).

The HE solubilized by the acetone–HCl solution (Table 4) showed elemental content similar to that of previous studies (Piccolo et al., 1998). The data indicate higher C and lower N values than for the humic fractions extracted with alkaline solutions. The C and H contents in HE were substantially reduced for all sites when passing from forested to cultivated soils with the exception of Sirinka for which the C content remained constant. However, except for Awassa, the C/H ratio increased at all sites with cultivation, suggesting a preferential persistence of condensed hydrocarbons in the residual HE fraction.

Isotopic measurements of SOC have been utilized successfully to document effects of land use changes on turnover rates of OM (Balesdent and Mariotti,

1996; Bernoux et al., 1998). The use of ^{13}C isotopic tracers for SOM studies is related to the discrimination against ^{13}C that all plants operate during photosynthesis but to different degrees which depend on their photosynthetic pathway (Farquhar et al., 1989). The isotopic $\delta^{13}\text{C}$ values shown in Table 3 indicate that at all locations OC is from C4 plants, given their generally lower values in the humic extracts. These values for the forested sites suggest a major vegetation change whereby the original C3 forests/forbs have been replaced or dominated by C4 grasses. According to Bernoux et al. (1998), terrestrial plants with C3 pathway (Calvin cycle) have $\delta^{13}\text{C}$ values in the range -35‰ to -20‰ whereas those with the C4 pathway (Hatch-Slack) have higher $\delta^{13}\text{C}$ values which range from -19‰ to -9‰ (Boutton, 1991). The higher (less negative) $\delta^{13}\text{C}$ values in both the forested and cultivated soils imply the dominance of C4 plants which was not affected by cultivation. Peterson and Fry (1987) and Nadelhoffer and Fry (1988) explained that the isotopic composition of SOM is identical with the isotopic composition of the vegetation from which it was derived because the fractionation during decomposition is small relative to the original fractionation during C fixation. This explains the $\delta^{13}\text{C}$ values found in these more humified OM fractions and agrees with the results of Spaccini et al. (2000) related to a European climatic sequence.

The isotopic $\delta^{13}\text{C}$ values of HE (Table 4) generally resembled those of the other humic fractions (Table 3) and can be explained by the same process described above. Flexor and Volkoff (1977), who studied changes in $\delta^{13}\text{C}$ values of humic fractions from an

Table 4
C, N and H contents (g kg⁻¹) and $\delta^{13}\text{C}$ values of humic extract (HE) solubilized with an acetone–HCl solution from forested (F) and cultivated (C) soils in Ethiopia

Location	Sites	C	N	H	C/N	C/H	$\delta^{13}\text{C}$
Awassa	F	609.8	20.2	47.7	30.2	12.8	-16.7
	C	386.7	10.8	34.4	35.8	11.2	-14.0
Ghinchi	F	633.3	12.3	60.6	51.5	10.4	-18.9
	C	591.0	11.4	47.0	51.8	12.6	-13.8
Holeta	F	502.7	10.0	49.9	50.3	10.1	-17.5
	C	371.1	10.0	26.8	37.1	13.8	-15.0
Jimma	F	607.1	18.2	53.4	33.4	11.4	-18.9
	C	380.2	17.3	27.1	21.9	14.0	-15.0
Sirinka	F	610.5	17.3	41.9	35.3	14.6	-13.5
	C	610.7	13.1	32.7	46.6	18.7	-14.3

Ultisol profile under Brazilian tropical forest, showed that the humic fractions had large negative $\delta^{13}\text{C}$ values. [Wedin et al. \(1995\)](#) observed the reverse phenomenon, similar to the one found in this study, and argued that the selective preservation of specific groups of compounds such as lignin accounted for the variable $\delta^{13}\text{C}$ pattern.

3.3. ^{13}C NMR spectra of humic fractions

Solid-state ^{13}C NMR spectra of HA, FA and HU from forested and cultivated soils are shown in [Figs. 1 and 2](#). The carbon distribution (intervals of chemical shift) in these humic fractions as measured from the NMR spectra are reported in [Table 5](#). Despite the quantitative error in the solid-state NMR technique, which was kept small by applying the VCT technique,

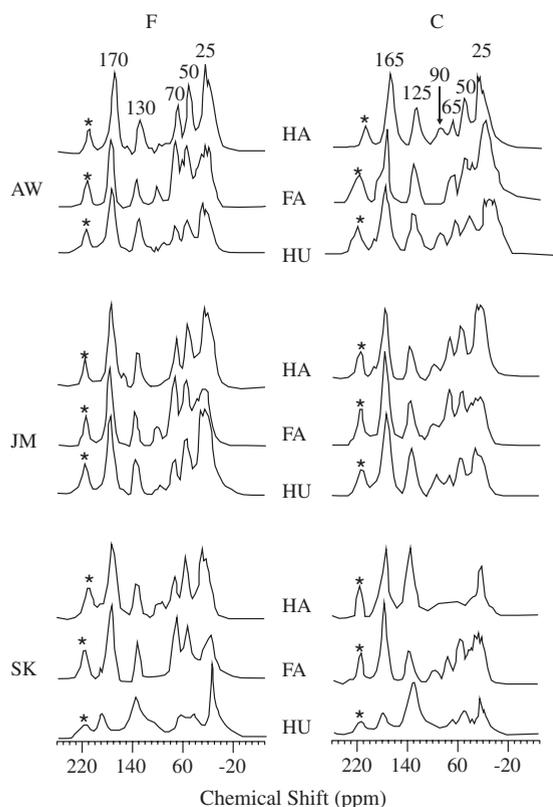


Fig. 1. CPMAS- ^{13}C NMR spectra of HA, FA and HU fractions extracted from Ethiopian soils (AW=Awassa; JM=Jimma; SK=Sirinka) at forested (F) and cultivated (C) sites. Spinning side bands (SSB) are indicated by an asterisk.

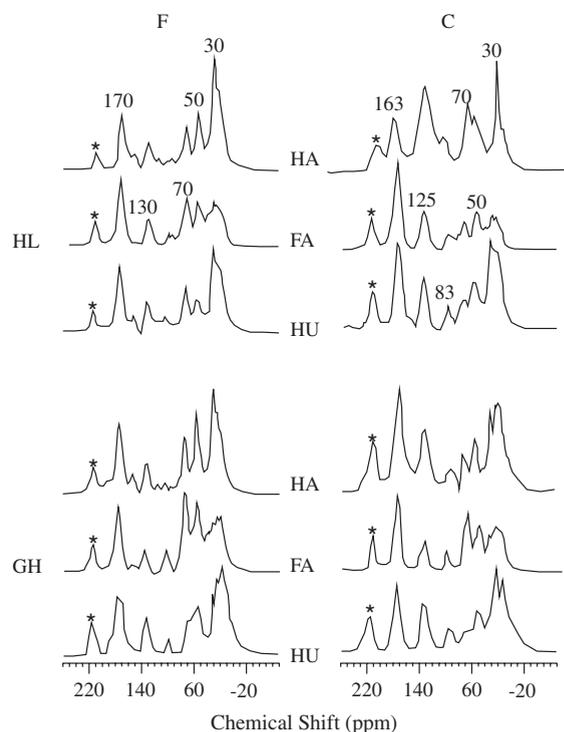


Fig. 2. CPMAS- ^{13}C NMR spectra of HA, FA and HU fractions extracted from Ethiopian soils (HL=Holeta; GH=Ginchi) at forested (F) and cultivated (C) sites. Spinning side bands (SSB) are indicated by an asterisk.

the spectra of HA, FA and HU showed that humic chemical composition varied with land use. At Awassa, no difference was observed in the alkyl region (0–50 ppm) of HA between the forested and cultivated soils, whereas the same region increased by 41% in FA and 31.8% in HU from forested to cultivated soil ([Table 5](#)). At Ghinchi, Holeta and Jimma, cultivation reduced the alkyl groups relative to the forested sites in all humic fractions, whereas at Sirinka the alkyl region decreased in HA and HU but slightly increased in FA as a result of cultivation ([Figs. 1 and 2](#)).

At Awassa, the carbonyl-C (160–200 ppm) for the cultivated soil showed an increasing trend for HA and FA and a decreasing one for HU, in respect to the forested soil ([Table 5](#)). At Ghinchi, Holeta and Sirinka, carbonyl-C increased due to cultivation in all humic fractions, whereas at Jimma it increased in FA and HU but decreased in HA as a result of deforestation. The increase in the carbonyl-C of the majority of these soils with cultivation may be

Table 5

Distribution of 13-carbon (%) in chemical shift intervals of ^{13}C NMR spectra of humic fractions (HA, FA, HU) from forested (F) and cultivated (C) sites in Ethiopia

Location	Sample	Site	Chemical Shift Intervals (ppm)					HI ^a	HB ^b	HB/HI ^c
			0–50	50–85	85–105	105–160	160–200			
Awassa	HA	F	32.0	12.7	12.0	6.0	37.3	62.0	38.0	0.61
		C	32.0	12.3	7.4	8.2	40.2	59.8	40.2	0.67
	FA	F	31.3	17.2	16.4	3.0	32.1	65.7	34.3	0.52
		C	44.2	11.5	8.8	0.0	35.4	55.8	44.2	0.79
	HU	F	25.5	11.1	9.6	2.9	51.0	71.6	28.4	0.40
		C	33.6	10.5	6.3	6.3	43.4	60.1	39.9	0.66
Ghinchi	HA	F	34.2	14.3	12.2	3.6	35.7	62.2	37.8	0.61
		C	33.5	7.4	17.4	5.4	36.4	61.2	38.8	0.64
	FA	F	28.6	17.9	20.1	4.5	29.0	67.0	33.0	0.49
		C	26.9	11.0	16.5	3.8	41.8	69.2	30.8	0.44
	HU	F	39.5	12.6	6.5	3.3	38.1	57.2	42.8	0.75
		C	27.5	7.4	9.0	5.3	50.8	67.2	32.8	0.49
Holeta	HA	F	43.2	12.2	12.8	2.7	29.1	54.1	45.9	0.85
		C	37.2	7.7	9.6	3.2	42.3	59.6	40.4	0.68
	FA	F	25.9	14.7	15.5	5.6	38.4	68.5	31.5	0.46
		C	21.1	11.8	8.1	3.7	55.3	75.2	24.8	0.33
	HU	F	39.4	10.8	9.4	4.4	36.0	56.2	43.8	0.78
		C	22.3	9.1	13.6	8.6	46.4	69.1	30.9	0.45
Jimma	HA	F	35.7	15.0	12.1	1.4	35.7	62.9	37.1	0.59
		C	34.5	12.1	14.3	4.5	34.5	61.0	39.0	0.64
	FA	F	27.8	15.9	20.5	6.0	29.8	66.2	33.8	0.51
		C	20.5	13.8	14.3	8.1	43.3	71.4	28.6	0.40
	HU	F	38.6	10.5	8.5	2.6	39.9	58.8	41.2	0.70
		C	20.9	10.2	7.3	6.8	54.8	72.3	27.7	0.38
Sirinka	HA	F	26.5	12.7	10.5	5.5	44.8	68.0	32.0	0.47
		C	22.8	1.6	7.9	7.1	60.6	70.1	29.9	0.43
	FA	F	25.3	15.7	16.1	2.3	40.6	72.4	27.6	0.38
		C	27.5	13.8	9.4	5.0	44.4	67.5	32.5	0.48
	HU	F	31.6	10.0	9.5	8.9	40.0	59.5	40.5	0.68
		C	18.1	10.1	7.2	9.1	55.4	72.8	27.2	0.37

^a HI=Percent of hydrophilic carbon: [(50–85)+(85–105)+(105–160)].

^b HB=Percent of hydrophobic carbon: [(0–50)+(160–200)].

^c HB/HI=Degree of hydrophobicity.

attributed to either the side-chain oxidation of plant-derived lignin–phenolic compounds (Guggenberger et al., 1995; Zech and Guggenberger, 1996) or incorporation of carbonyl-rich material from fresh vegetal tissues (Spaccini et al., 2000).

Signals relative to aromatic carbons (105–160 ppm) were generally increased in HA and HU but decreased in FA passing from forested to cultivated soils, with the exception of Jimma and Sirinka which showed an increase in this region for all humic fractions. This suggests a selective accumulation of aromatic carbons through intimate adsorption on the finest soil particles in the case of HU (Piccolo, 1996) and a progressive oxidation through a microbially

mediated opening of aromatic rings passing from HA to FA (Stevenson, 1994).

With cultivation, the hydrophilic carbon (HI) in HA increased at Holeta and Sirinka but decreased at Jimma and Awassa and was about the same at Ghinchi (Table 5). The HI in FA also decreased as a result of cultivation at Awassa and Sirinka but increased at Jimma, Ghinchi and Holeta, whereas that of HU decreased at Awassa and increased substantially in all other sites. The hydrophobic carbon (HB) appeared to generally decrease, with the exception of Awassa, when passing from forested to cultivated sites. The degree of hydrophobicity (HB/HI) varied among humic fractions and between forested and cultivated

soil in each location (Table 5). When the HB/HI values were averaged between forested and cultivated soils in each location, it was evident that cultivation clearly affected the values by increasing at Awassa and generally decreasing at all other sites.

A review of the factors controlling OM dynamics in tropical soils has been reported by Zech et al. (1997). They claimed that changes in OM during the process of decomposition are affected by the dominant site-specific characteristics like temperature, soil moisture content, pH and nutrient levels. Our results indicate also an influence of land use on the supposed stable humified pool of SOM. The signals due to alkyl-C in the humic fractions extracted by the traditional alkaline solutions (Table 5) decreased substantially from the forested to the cultivated soils with the exception of the Awassa Andisol. The C–O and C–N region (50–85 ppm) decreased at most sites, while a concomitant increase in the anomeric-C in carbohydrates (105 ppm) was generally observed. Kögel-Knabner et al. (1992) suggested that mobile alkyl components were relatively rapidly degraded in temperate soils, whereas they attributed a selective preservation of other alkyl groups in more rigid structures to their larger size or degree of cross-linking. The increase of carbohydrate C in humic fractions of cultivated soils is a consequence of the microbial degradation of crop residues and the incorporation of released carbohydrates into the humic structures (Spaccini et al., 2000). Our findings reveal that cultivation led to a rapid modification of humified organic matter in these tropical soils, mainly resulting in a loss of C–O and C–N (50–85 ppm) containing material and increasing dominance of C-substituted, recalcitrant, aromatic structures with signals near 130 ppm.

The different behaviour of the Awassa Andisol in the changes of humic fractions with cultivation may be ascribed to its andic character and its large amounts of allophanes and halloysite. These inorganic components favour the accumulation of SOM (Table 1) that subsequently decomposes or is altered at slower rates than other soils. Phosphate limitation and adsorption of organic compounds have been adjudged as mechanisms for the stabilization of SOM in soils (Zech et al., 1997). The SOM fixed by Andisols is poorly altered by microbial oxidation and becomes preferentially richer in alkyl and aromatic constituents than in O-alkyl species (Conte et al., 1997; 2003). The

large aromaticity of Andisols may additionally contribute to the stability of their SOM.

The ^{13}C NMR spectra of HE fractions are shown in Fig. 3, whereas the C distribution calculated from the spectra are given in Table 6. A large alkyl signal in the 0–50 ppm region is evident in the spectra of all soils (Fig. 3). This is in line with previous studies (Piccolo et al., 1990, 1998; Spaccini et al., 2000) which indicated a large apolar character of this fraction in the humic pool of soils. The strong signal in the alkyl region centered at around 25 ppm (Fig. 3) is commonly attributed to CH_2 and CH_3 groups in polymethylene chains (Piccolo et al., 1998).

Oades et al. (1987) and Baldock et al. (1992) showed that alkyl compounds constitute the basic

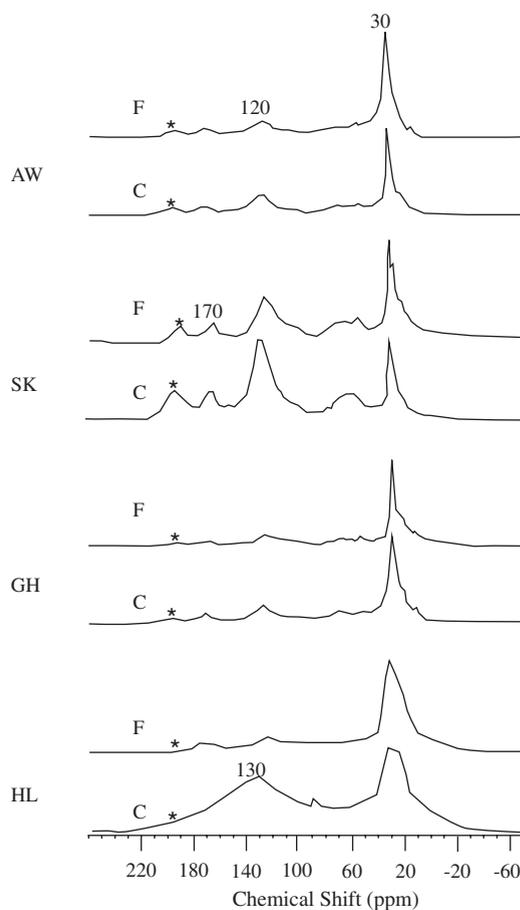


Fig. 3. CPMAS- ^{13}C NMR spectra of HE fractions extracted from Ethiopian soils (AW=Awassa; SK=Sirinka; GH=Ginchi; HL=Holeta) at forested (F) and cultivated (C) sites. Spinning side bands (SSB) are indicated by an asterisk.

Table 6

Distribution of 13-carbon (%) in chemical shift intervals of ^{13}C NMR spectra of humic extracts (HE) solubilized with an acetone–HCl solution from forested (F) and cultivated (C) sites of Ethiopia

Location	Sites	Chemical Shift Intervals (ppm)					HI ^a	HB ^b	HB/HI ^c
		0–50	50–85	85–105	105–160	160–200			
Awassa	F	48.4	19.3	0.5	15.4	16.5	36.3	63.7	1.76
	C	42.7	16.8	0.2	17.1	23.2	40.2	59.8	1.49
Ghinchi	F	54.3	17.0	0.3	13.6	14.8	32.1	67.9	2.11
	C	46.8	20.0	0.4	15.6	17.0	37.4	62.4	1.67
Holeta	F	59.2	14.0	0.4	18.3	8.1	22.5	77.5	3.44
	C	33.5	17.0	0.2	29.4	20.0	37.2	62.8	1.69
Sirinka	F	35.4	19.0	0.4	24.3	20.8	40.2	59.7	1.48
	C	20.8	17.3	0.3	29.4	32.2	49.8	50.2	1.01

^a HI=Percent of hydrophilic carbon: [(50–85)+(85–105)+(105–160)].

^b HB=Percent of hydrophobic carbon: [(0–50)+(160–200)].

^c HB/HI=Degree of hydrophobicity.

component of the recalcitrant OM strictly associated with the finest ($< 2 \mu\text{m}$) soil particles. The HE fractions of this study show very similar chemical characteristics as the most resistant OM fraction isolated from surface horizons of forested soils after sequential extraction and vigorous hydrolysis of the resulting humin residues as reported by Kögel-Knabner et al. (1992) and Augris et al. (1998). These authors suggested that the non-hydrolysable, highly alkyl organic residue may consist of preserved macromolecular materials directly inherited from higher plants. The alkyl structures present in the HE fraction are likely to represent the most persistent fraction of stable OM in soils based on a mechanism of selective preservation of hydrophobic components in soils (Lichtfouse et al., 1998; Piccolo, 2002). The stability of the HE material in soils may be attributed to the entropy-driven separation of this hydrophobic fraction from water and, in turn, from microbial activity (Conte and Piccolo, 1999; Piccolo, 2002).

The hydrophobic domain (aromatic-C+aliphatic-C) of HE spectra varied from 50.2% in cultivated Sirinka to 77.5% in forested Holeta (Table 6). This large content of hydrophobic material resulted in a higher HB/HI ratio than for the HA, FA and HU fractions (Table 5). In all sites, cultivation caused a decrease of hydrophobic groups. With respect to the different content of C (Table 6), the change from forested into cultivated soils produced a decrease in the alkyl-C (0–50 ppm) with concomitant increase in both aromatic- and carbonyl-C groups (105–200 ppm). The carbons in the 50–85 and 85–105 ppm

ranges decreased in Awassa and Sirinka but increased in Ghinchi and Holeta due to cultivation.

The decrease of HE with cultivation agrees with the general diminution of alkyl components observed also for the HA, FA and HU fractions (Table 5). This finding suggests that the profound structural alteration when a tropical forested soil is increasingly disturbed by cultivation practices results in significant chemical changes of the nature of humic matter. The humic substances of the tropical soils of this study were not only decreased in quantity with cultivation but lost preferentially their alkyl constituents, whereas the relative content of aromatic and oxidized carbon was concomitantly enhanced. Previous works suggested that the hydrophobic components of HE are essential to the preservation and stability of OM in soils (Spaccini et al., 2000). Moreover, it has been found that the larger the hydrophobicity of humic substances in soil, the lower is the mineralization of biolabile compounds and the release of CO_2 (Piccolo et al., 1999). The loss of the most hydrophobic fraction due to disruption of soil macroaggregates and exposure of organic compounds to biotic and abiotic oxidation is conducive to less stable humic associations and more fragile soil structures (Piccolo, 2002). The humic matter resulting from deforestation and cultivation of these Ethiopian soils became relatively richer in either the hydrophilic, more oxidized fulvic components, or the HU fraction, that remains protected in the structurally strong soil microaggregates. These results are in line with those of Piccolo and Mbagwu (1999) which showed that the hydro-

phobic constituents of SOM are responsible for the long-term stabilization of soil structure such as that found in undisturbed soil under forest. The degradation of soil structure occurring with cultivation and the consequent phenomena of severe erosion in tropical soils (Mbagwu and Piccolo, 1998) may then be related to the progressive loss of the cementing action of alkyl compounds in soil.

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