

# Soil Organic Matter Dynamics in the Subhumid Agroecosystems of the Ethiopian Highlands: Evidence From Natural $^{13}\text{C}$ Abundance and Particle-Size Fractionation

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## ABSTRACT

We used natural  $^{13}\text{C}$  abundance coupled with particle-size fractionation to evaluate soil organic carbon (SOC) dynamics following deforestation and subsequent cultivation in the subhumid Ethiopia highlands. Surface soil (0–10 cm), leaf, root, and litter samples were collected from natural forest and fields cultivated for 25 yr (Wushwush) and from *Podocarpus* dominated natural forest and 30 yr cultivated fields (Munesa) and C, N and  $\delta^{13}\text{C}$  signature were measured. Total SOC declined by 55% ( $32.0 \text{ Mg ha}^{-1}$ ) at Wushwush and by 63% ( $40.2 \text{ Mg ha}^{-1}$ ) at Munesa following cultivation, while losses of N amounted to 52% ( $2.8 \text{ Mg ha}^{-1}$ ) and 60% ( $3.1 \text{ Mg ha}^{-1}$ ) at the two sites, respectively.  $\delta^{13}\text{C}$  values of bulk soils of natural forests at Wushwush ( $-24.3\text{‰}$ ) and Munesa ( $-23.4\text{‰}$ ) were significantly lower than those from the corresponding cultivated fields ( $-19.9\text{‰}$ , Wushwush and  $-15.5\text{‰}$ , Munesa). Deforestation and continuous cultivation at Wushwush and Munesa resulted in depletion of 80 and 96% of the initial forest-derived SOC in sand, while 73 and 85% of  $\text{C}_3$  SOC was lost from silt fraction of the two sites, respectively. These results suggest that SOC in sand was a very labile component of SOM and is a more sensitive indicator to changes in soil C storage in response to land use changes. However, the substantial amount of forest-derived SOC lost from silt indicates that SOM associated with silt was also quite susceptible to management changes, and that at least in the soils under study represents a moderately labile SOM pool, which is generally not the case in temperate soils. Forest-derived SOC in clay declined by 48 and 61% at Wushwush and Munesa, respectively, suggesting that clay retained  $\text{C}_3$  derived SOC more effectively and that SOM bound to clay was more stable than SOM associated with sand and silt fractions.

SOIL ORGANIC MATTER is a large reservoir of C that can act as a sink or source of atmospheric  $\text{CO}_2$  (Lugo and Brown, 1993). It is also an important source of inorganic nutrients for plant production in natural and managed ecosystems. Moreover, SOM governs structural stability and cation exchange capacity of soils either directly, through its chemical structure and surface properties, or indirectly as a source of energy and nutrients for soil biota (Zech et al., 1997). These effects are especially important in cultivated tropical soils, where SOM is frequently related to soil fertility and productivity.

Replacement of natural forests by agroecosystems in the subhumid and humid tropics generally increases the flux of terrestrial C to the atmosphere, reduces levels of SOM, and thereby decreases soil fertility. Therefore, development of sustainable agricultural systems

requires analytical methods that can (i) accurately monitor changes in the amount, nature, and dynamics of SOM pools in natural and human-influenced systems and (ii) estimate C fluxes between the soils and atmosphere (Bernoux et al., 1998).

Various chemical fractionation and characterization methods have been used to describe SOM dynamics (Stevenson et al., 1989; Christensen, 1992). However, these methods have not proven particularly useful in following the dynamics of organic materials in soils and in identifying specific SOM pools that diminish upon intensive management (Stevenson et al., 1989). This is in part due to the complex nature of SOM and to the enormous array of compounds that exists in the soil, ranging from recent plant materials through a continuum of metabolic products of microorganisms to components of stable humus (Stevenson et al., 1989; Zech et al., 1997). Recently, biologically meaningful fractions or pools have been obtained by methods based on physical fractionation of soil according to particle-size separates without chemical treatments, which combined with biological and chemical analysis allows further insight into the functional attributes of the size separates (Tieszen and Stewart, 1983; Bond et al., 1992; Guggenberger et al., 1995; Lehmann et al., 1998; Solomon et al., 2000). Moreover, the use of  $^{13}\text{C}$  natural abundance technique coupled with particle-size fractionation have further advanced SOM turnover studies, since these methods are well suited to study soil organic carbon (SOC) dynamics over a time scale ranging from a few to several hundred years and are relevant for understanding the consequences of natural and anthropogenic vegetation changes (Balesdent et al., 1990; Boutton et al., 1998; Koutika et al., 2000; Shang and Tiessen, 2000; Gerzabek et al., 2001). The usefulness of  $^{13}\text{C}$  isotopic tracers for SOM studies derives from the contrasting metabolic pathways of  $\text{C}_3$  and  $\text{C}_4$  plants (O'Leary, 1988; Farquhar et al., 1989). The isotopic composition of SOM closely resembles the isotopic composition of the vegetation from which it has been derived because the fractionation that occurs during decomposition is small compared with the original fractionation during C fixation (Ågren et al., 1996). Thus, growing  $\text{C}_4$  plants on a soil previously supporting  $\text{C}_3$  vegetation can be considered as an in situ labeling of newly incorporated organic matter into the soil.

Extensive deforestation and conversion of natural forests into agricultural land is the most wide spread change in land use in Ethiopian highlands. In the past

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**Abbreviations:** ANOVA, analysis of variance;  $\text{C}_{\text{dnf}}$  and  $\text{C}_{\text{dmc}}$ , organic C derived from natural forest and maize crop residues; CEC, cation exchange capacity;  $\text{C}_{\text{ms}}$  and  $\text{C}_{\text{fs}}$ , total SOC in the cultivated and forest soils or size separates;  $\delta_{\text{ms}}$  and  $\delta_{\text{f}}$ ,  $\delta^{13}\text{C}$  value of fresh leaves and roots of maize crop and forest vegetation;  $\delta_{\text{ms}}$  and  $\delta_{\text{fs}}$ ,  $\delta^{13}\text{C}$  values of bulk soils or size separates of the cultivated fields and natural forests; LSD, least significant difference; SOC, soil organic carbon; SOM, soil organic matter; V-PDB, Vienna-Pee Dee Belemnite limestone;  $r_b$ , bulk density.

100 yr, the total area of land covered by the forest in Ethiopia has declined from about 40% to an estimated 2.4% in 1990 (Pohjonen and Pukkala, 1990; Eshetu and Högborg, 2000). Forest degradation and destruction has continued unabated especially in south and southwestern parts of the country, where most of the remnant subhumid tropical highland forests are found. This has led to extensive environmental degradation that threatens the fragile ecosystems of the region. A considerable portion of the cleared area has been put under continuous maize (*Zea mays* L.) cultivation. This vegetation change provided a unique opportunity to study SOM dynamics in different pools on the basis of a progressive shift in isotopic composition of the SOC following the replacement of natural forests ( $C_3$  species) by maize crop ( $C_4$  species) in subhumid tropical highland environments.

The objectives of this study are (i) to assess SOC turnover in bulk soils and particle-size separates (sand, silt, and clay) by means of natural  $^{13}C$  isotope tracer techniques and (ii) to quantify the total SOC and N losses following deforestation and continuous maize cultivation in the subhumid tropical highland agroecosystems of southern Ethiopia.

## MATERIALS AND METHODS

### Site Description

The study was conducted at the southwestern highlands (Wushwush) and the southeastern Rift Valley escarpments (Munesa) of Ethiopia. Wushwush is located at  $7^{\circ} 19' N$  and  $36^{\circ} 07' E$ . The altitude of the area is 1900 m above sea level. Mean annual temperature is  $18^{\circ}C$  with an average annual precipitation of 1800 mm. Geologically, the area is associated with Jimma Volcanics, with abundant rhyolites and trachybasalts. The soils of the area are classified as plinthic Paleudalf with clayey texture and dark reddish brown color (Soil Survey Staff, 1999). The Wushwush natural forest is mainly composed of *Olea africana* Mill., *Syzygium guineense* Guill. ex Perr., *Cordia africana* Lam., *Croton macrostachys* Hochst. ex A. Rich., and *Ficus vasta* Forsk. The Munesa site is located at  $7^{\circ} 35' N$  and  $38^{\circ} 45' E$ . Mean annual temperature is  $19^{\circ}C$  with an average annual precipitation of 1250 mm. Parent materials of the Munesa area are of volcanic origin, principally trachytes and basalts with ignimbrites and pumices at the rift valley floor. The escarpment extends from about 2100 m to 3200 m and the plain descends gradually to the Rift Valley lakes at about 1600 m above sea level. The soils of the area are classified as Typic Palehumults with clayey texture and very dark reddish brown color (Soil Survey Staff, 1999). The natural vegetation of the Munesa forest ranges from *Arundinaria alpina* K. Schun., *Hagenia abyssinica* Bruce ex J.F. Gmel., *Croton macrostachys* Hochst. ex Rich., *Podocarpus falcatus* Thunb. ex Mirb., *Olea hochstetteri* Baker. dominated forest on the escarpment to *Acacia* woodlands (*Acacia tortilis* Forssk. ex Hayne, *Acacia abyssinica* Hochst. ex Benth., and *Acacia seyal* Delile) in the semiarid lowlands.

The land use systems studied at the Wushwush site were natural forest ( $C_3$ ) and fields cultivated continuously with maize for 25 yr ( $C_4$ ), while at the Munesa site, *Podocarpus* dominated natural forest ( $C_3$ ) and fields cultivated continuously with maize for 30 yr ( $C_4$ ) were investigated. In the cultivated fields of both sites, maize was grown without fertilizer inputs. However, during the intermittent dry periods, sorghum [*Sorghum bicolor* (L.) Moench] was grown at Munesa. The depth of ploughing at the Wushwush and Munesa sites

varies between 10 to 12 cm. Crop residues that remain on the cultivated fields are normally collected and used as animal feed.

After considering the depth of cultivation in the region, we used a core sampler (200-cm<sup>3</sup> core volume at each subsite) and collected soil samples in three replicates from the upper 10 cm of the different land use systems in April 1998. This helps to minimize differences, which may arise because of the dilution of SOM by mixing the surface soil with that of the subsoil through ploughing. We selected three representative fields from each land use system and collected nine subsamples in a radial sampling scheme from each site (Wilding, 1985). A composite sample was then prepared from the subsamples. The samples were air dried and sieved (<2 mm) prior to fractionation and chemical analysis. In addition, fresh leaf, root, and undecomposed and decomposed litter samples were collected from the natural forest sites, whereas only fresh leaves and roots were collected from the fields cultivated with maize.

### Particle-Size Fractionation

Particle-size fractionation was done on <2-mm material (bulk soil) according to Amelung et al. (1998). After removing visible root remnants, 30 g of soil was treated ultrasonically with an energy input of 60 J mL<sup>-1</sup> by a probe type sonicator (Branson Sonifier W-450, Danbury, CT) in a soil:water ratio of 1:5 (w/v). Coarse sand size fraction (250–2000  $\mu m$ ) was isolated by wet sieving. To completely disperse the remaining material in the <250- $\mu m$  suspension, ultrasound was again applied with an energy input of 440 J mL<sup>-1</sup> in a soil:water ratio of 1:10 (w/v). The clay size fraction (<2  $\mu m$ ) was separated from the silt (2–20  $\mu m$ ) and fine sand (20–250  $\mu m$ ) size fractions by repeated centrifugation. The silt size fraction was separated from the fine sand fraction by wet sieving. Coarse and fine sand fractions were combined and finally all size separates were dried at  $40^{\circ}C$  before grinding them for chemical analysis. The recovery of size separates after ultrasonic dispersion, wet sieving, and centrifugation ranged from 97 to 98% of the initial soil mass.

### Chemical Analysis

Carbon and N contents were analyzed by dry combustion with a C/H/N/S-analyzer (Elementar Vario EL, Hanau, Germany). The pH-H<sub>2</sub>O and pH-KCl were determined in 1:2.5 soil:water suspension with a pH meter. Cation-exchange capacity (CEC) was determined by means of 1 M NH<sub>4</sub>OAc (pH = 7.0) according to Avery and Bascomb (1974). Dithionite-citrate-bicarbonate extractable aluminum and iron (Al<sub>d</sub>, Fe<sub>d</sub>) were determined after two extractions at  $70^{\circ}C$  for 15 min as described by Mehra and Jackson (1960). Oxalate-extractable aluminum and iron (Al<sub>o</sub>, Fe<sub>o</sub>) were determined after extraction for 2 h by means of 0.2 M ammonium oxalate at pH = 3 in the dark (Blume and Schwertmann, 1969) (Table 1).

Natural  $^{13}C$  abundance was determined with an elemental analyzer (Carlo Erba EL 1108, Hanau, Germany) coupled with an isotope ratio mass spectrometer (Finningan MAT Delta S, Bremen, Germany) via a ConFlo II Interface. The C isotope ratios of the samples were compared with the isotope ratio of reference CO<sub>2</sub> gas (99.995% purity), calibrated against V-PDB; the international standards NBS 19 and sucrose ANU were used as references from the National Bureau of Standards (USA) and the International Atomic Energy Agency (Austria). The analytical precision of  $\delta^{13}C$  measurements was 0.12‰.

### Calculation of C Derived from $C_3$ and $C_4$ Vegetation

Stable  $^{13}C$  isotope abundance is commonly expressed by  $\delta$  notation in per mil (‰), as the relative deviation in the ratio

**Table 1. Physical and chemical characteristics of surface soils (0–10 cm) in the sub-humid highlands of southern Ethiopia.**

Land use	Bulk density	pH		CEC	BS	Fe <sub>d</sub> †	Fe <sub>o</sub> †	Al <sub>d</sub> †	Al <sub>o</sub> ‡
	Mg m <sup>-3</sup>	H <sub>2</sub> O	KCl	cmol kg <sup>-1</sup> soil	%	g kg <sup>-1</sup> soil			
<b>Wushwush</b>									
Natural forest	0.69 (0.05)§	6.4 (0.08)	5.5 (0.05)	37.0 (0.11)	69 (0.01)	6.7 (0.05)	0.60 (0.02)	0.96 (0.04)	0.25 (0.05)
Cultivation	0.89 (0.08)	5.7 (0.05)	4.7 (0.04)	26.4 (0.15)	46 (0.05)	5.8 (0.01)	0.83 (0.02)	0.77 (0.03)	0.30 (0.07)
<b>Munesa</b>									
Natural forest	0.62 (0.10)	7.6 (0.05)	6.8 (0.07)	52.2 (0.12)	98 (0.12)	2.5 (0.03)	0.30 (0.02)	0.38 (0.04)	0.15 (0.01)
Cultivation	1.04 (0.06)	5.6 (0.09)	4.5 (0.05)	26.5 (0.05)	51 (0.01)	3.4 (0.03)	0.71 (0.08)	0.40 (0.03)	0.21 (0.05)

† Fe<sub>d</sub> and Al<sub>d</sub>, dithionite-citrate-bicarbonate extractable Fe and Al.

‡ Fe<sub>o</sub> and Al<sub>o</sub>, oxalate extractable Fe and Al.

§ Values in parentheses indicate standard errors of the mean ( $n = 3$ ).

of the heavy isotope to the light isotope in a sample ( $^{13}\text{C}/^{12}\text{C}_{\text{sam}}$ ) compared with that of a standard ( $^{13}\text{C}/^{12}\text{C}_{\text{std}}$ ):

$$\delta^{13}\text{C} (\text{‰}) = \left[ \frac{\left(\frac{^{13}\text{C}}{^{12}\text{C}}\right)_{\text{sam}} - \left(\frac{^{13}\text{C}}{^{12}\text{C}}\right)_{\text{std}}}{\left(\frac{^{13}\text{C}}{^{12}\text{C}}\right)_{\text{std}}} \right] \cdot 1000 \quad [1]$$

The standard used for reporting  $^{13}\text{C}$  data ( $^{13}\text{C}/^{12}\text{C} = 0.0112372$ ) is the Vienna-Pee Dee Belemnite limestone (V-PDB) (Coplen, 1995).

Organic C derived from natural forest ( $C_{\text{dnf}}$ ) and maize crop residues ( $C_{\text{dmc}}$ ) was expressed either as g SOC kg<sup>-1</sup> soil or as percentage of the total SOC ( $C_{\text{dnf}}[\%]$  and  $C_{\text{dmc}}[\%]$ ) in the cultivated fields. These SOC pools were calculated by the following equations:

$$C_{\text{dmc}} = C_{\text{ms}} \cdot \frac{(\delta_{\text{ms}} - \delta_{\text{fs}})}{(\delta_{\text{m}} - \delta_{\text{f}})} \quad C_{\text{dnf}} = C_{\text{ms}} - C_{\text{dmc}} \quad [2]$$

$$C_{\text{dmc}}[\%] = \frac{(\delta_{\text{ms}} - \delta_{\text{fs}})}{(\delta_{\text{m}} - \delta_{\text{f}})} \cdot 100 \quad C_{\text{dnf}}[\%] = 100 - C_{\text{dmc}}[\%] \quad [3]$$

where  $C_{\text{ms}}$  denotes total SOC content of samples from the cultivated soils or separates, while  $\delta_{\text{ms}}$  and  $\delta_{\text{fs}}$  denote average  $\delta^{13}\text{C}$  values of the respective soils or size separates from the cultivated fields and natural forests, respectively. The  $\delta_{\text{m}}$  and  $\delta_{\text{f}}$  represent the average  $\delta^{13}\text{C}$  values of fresh leaves and roots of maize crop and forest vegetation, respectively.

Theoretically, after a vegetation change from forest ( $C_3$ ) to maize crop ( $C_4$ ),  $\delta^{13}\text{C}$  values of a soil supporting only the original forest vegetation and  $\delta^{13}\text{C}$  values of a soil supporting only maize crop have to be also measured to calculate the amounts ( $C_{\text{dnf}}$  and  $C_{\text{dmc}}$ ) and percentages of SOC derived from forest and maize vegetation ( $C_{\text{dnf}}[\%]$  and  $C_{\text{dmc}}[\%]$ ); Eq. [2] and [3] are used for this. The  $\delta^{13}\text{C}$  value of the soil supporting only forest vegetation can be obtained from the ecological reference site with similar soil type and topography kept under the original forest vegetation. However, it is not possible to measure the equilibrium  $\delta^{13}\text{C}$  value of soil supporting only maize, since there is no site that has been kept only under maize cultivation for a sufficient period that it can be used as an ecological reference site for  $C_4$  vegetation. Since such a reference sites does not exist, Balesdent and Mariotti (1987) and Van Noordwijk et al. (1997) stated that the difference between  $\delta^{13}\text{C}$  values of a soil supporting maize and forest vegetation [ $\text{Dd} = \delta^{13}\text{C}(\text{soil only under maize}) - \delta^{13}\text{C}(\text{soil only under forest})$ ] must be estimated. Thus, several researchers substituted the  $\delta^{13}\text{C}$  value of soil supporting only the final  $C_4$

vegetation with the  $\delta^{13}\text{C}$  values of fresh leaves, roots, or litter derived from the final  $C_4$  vegetation to estimate the difference (Balesdent et al., 1988; Vitorello et al., 1989; Desjardins et al., 1994). Since isotopic enrichment can occur during decomposition of organic matter (Balesdent et al., 1990), the C remaining after mineralization is generally richer in  $^{13}\text{C}$  than the original plant material. Thus, the above assumptions involve comparison of materials at different states of humification. Bernoux et al. (1998) suggested that the difference in  $\delta^{13}\text{C}$  values of the final ( $C_4$ ) and original vegetation ( $C_3$ ) [ $\text{Dd} = \delta^{13}\text{C}(\text{final Veg.}) - \delta^{13}\text{C}(\text{initial Veg.})$ ] can be used to approximate the difference in  $\delta^{13}\text{C}$  values of a soil supporting the initial ( $C_3$ ) and final ( $C_4$ ) vegetation [ $\text{Dd} = \delta^{13}\text{C}(\text{soil only under final Veg.}) - \delta^{13}\text{C}(\text{soil only under initial Veg.})$ ]. This approach might help to minimize the error associated with comparing organic materials at different state of humification to approximate the potential difference in  $\delta^{13}\text{C}$  values of soils kept only under  $C_3$  and  $C_4$  vegetation. Thus, in the present study, the difference between mean  $\delta^{13}\text{C}$  value of fresh leaves and roots of maize crop ( $\delta_{\text{m}} = -12.2\text{‰}$  at both sites) and forest vegetation ( $\delta_{\text{f}} = -30.1\text{‰}$  and  $-26.6\text{‰}$  at Wushwush and Munesa, respectively) were used in Eq. [2] and [3] to calculate SOC derived from  $C_3$  and  $C_4$  vegetation (Tables 2 and 3).

Loss of forest-derived SOC as a result of deforestation and subsequent cultivation ( $\text{Loss}(C_{\text{dnf}})$  [%]) was calculated as follows:

$$\text{Loss}(C_{\text{dnf}})[\%] = 100 - C_{\text{dnf}}[\%] \cdot \left(\frac{C_{\text{ms}}}{C_{\text{fs}}}\right) \quad [4]$$

where  $C_{\text{ms}}$  and  $C_{\text{fs}}$  denotes total SOC in the cultivated and forest soils or size separates.

**Table 2. Amounts of SOC derived from natural forest ( $C_{\text{dnf}}$ ) and maize crop residues ( $C_{\text{dmc}}$ ) and losses of forest-derived SOC ( $\text{Loss}(C_{\text{dnf}})$ ) in bulk soils of two cultivated fields in southern Ethiopian highlands.**

	SOC contents		SOC stocks	
	Wushwush	Munesa	Wushwush	Munesa
	g kg <sup>-1</sup> soil		Mg ha <sup>-1</sup>	
$C_{\text{dnf}}^\dagger$	28.7 (0.08)§‡	17.1 (0.1)	19.8†	10.6 (0.06)
$C_{\text{dmc}}^\dagger$	9.4 (0.05)	21.1 (0.03)	6.5 (0.05)	13.1 (0.02)
$\text{Loss}(C_{\text{dnf}})^\dagger$	55.7 (0.06)	86.0 (0.09)	38.5 (0.08)	53.3 (0.04)

†  $C_{\text{dnf}}$ , Carbon-derived from natural forest;  $C_{\text{dmc}}$ , Carbon derived from maize crop residue;  $\text{Loss}(C_{\text{dnf}})$ , loss of natural forest-derived carbon.

‡ Corrected, the results were corrected for bulk density differences between the natural forest and the corresponding cultivated fields.

§ Values in parentheses indicate standard errors of the mean ( $n = 3$ ).



**Table 3. Amounts and proportions of SOC derived from natural forest and maize plant materials and losses of SOC derived from natural forests in particle-size separates of arable soils in the sub-humid highlands of southern Ethiopia.**

Fraction	SOC derived from forest		SOC derived from maize		Loss of forest-derived SOC	
	g kg <sup>-1</sup> soil	%	g kg <sup>-1</sup> soil	%	g kg <sup>-1</sup> soil	%
	<b>Wushwush</b>					
Sand	1.5 (0.07)†	61.0 (0.12)	0.9 (0.08)	39.0 (0.05)	9.3 (0.13)	87.0 (0.11)
Silt	8.7 (0.10)	75.0 (0.18)	2.9 (0.16)	25.0 (0.11)	22.1 (0.08)	72.0 (0.07)
Clay	21.7 (0.11)	92.0 (0.13)	1.9 (0.08)	8.0 (0.11)	18.4 (0.14)	46.0 (0.04)
	<b>Munesa</b>					
Sand	0.4 (0.03)	26.0 (0.10)	1.3 (0.02)	75.0 (0.18)	7.6 (0.13)	95.0 (0.05)
Silt	6.5 (0.06)	36.0 (0.08)	11.8 (0.09)	64.0 (0.05)	47.0 (0.03)	87.0 (0.04)
Clay	14.6 (0.05)	65.0 (0.07)	7.7 (0.05)	35.0 (0.10)	26.0 (0.08)	64.0 (0.05)

† Values in parenthesis indicate standard errors of the mean ( $n = 3$ ).

The total SOC stocks of the surface soils (Total SOC, Mg ha<sup>-1</sup>) were calculated from SOC contents of samples (g kg<sup>-1</sup> soil), soil layer thickness ( $z$ , m), and bulk density ( $\rho_b$ , Mg m<sup>-3</sup>), of the samples by the following equation:

$$\text{Total SOC} = \text{SOC} \cdot z \cdot \rho_b \cdot 10 \quad [5]$$

Forest clearing and cultivation usually cause compaction and consequently the bulk density of the cultivated soils increases with time. Therefore, samples of forest soils at a certain depth may not be directly comparable with samples of cultivated soils from the same depth. Since the bulk density has an important effect on C-balance calculations, Veldkamp (1994) suggested that when comparing total SOC contents of forested and cultivated or pasture soils, if sampling is based on depth, the depth of the soil layer has to be adjusted to avoid an error due to differences in bulk density. Thus, in the present study, the thickness of the cultivated soils ( $z$ ) was corrected ( $z_{\text{corrected}}$ ) as follows, assuming that the bulk density and depth of the cultivated soils were originally the same as those of the corresponding forest soils:

$$z_{\text{corrected}} = \left( \frac{\rho_{b \text{ forest}}}{\rho_{b \text{ cultivation}}} \right) \cdot z \quad [6]$$

### Statistical Analysis

Statistical analysis of the data was carried out on the replicates by one-way analysis of variance (ANOVA). If the main effects were significant at  $P < 0.05$ , a post hoc separation of means was done by univariate least significant difference (LSD) test. All statistical analyses were conducted with the software package Statistica 5.1 for Windows (1995).

## RESULTS AND DISCUSSION

### SOC and N Distribution in Bulk Soils and Particle-Size Separates

Deforestation of natural forests in conjunction with continuous cultivation induced a significant depletion of SOC and N in bulk soils and particle-size separates of these subhumid tropical highland agroecosystems. Total SOC and N contents in bulk soils of the cultivated fields at Wushwush and Munesa sites were significantly

**Table 4. Soil organic carbon (SOC) and N contents and C/N ratios bulk soils (0–10 cm) at two sites in southern Ethiopian highlands.**

Land use	SOC		N		SOC		N	
	g kg <sup>-1</sup> soil	C/N	Mg ha <sup>-1</sup>		Mg ha <sup>-1</sup>			
			uncorrected†	corrected‡	uncorrected†	corrected‡		
	<b>Wushwush</b>							
Natural forest	84.5a‡§	7.8a	10.8	58.3a	5.4a	58.3a	5.4a	
Cultivation	38.1b	3.7b	10.2	33.9b	3.3b	26.3b	2.6b	
	<b>Munesa</b>							
Natural forest	103.1a	8.2a	12.6	63.9a	5.1a	63.9a	5.1a	
Cultivation	38.2b	3.3b	11.8	39.7b	3.4b	23.6b	2.0b	

† Uncorrected, the results were not corrected for bulk density differences between the natural forest and the corresponding cultivated fields.

‡ Corrected, the results were corrected for bulk density differences between the natural forest and the corresponding cultivated fields.

§ Within one site, different letters along the column indicate significant differences between mean values of the different land use systems at  $P < 0.05$  ( $n = 3$ ).

lower ( $P < 0.05$ ) than the corresponding natural forests (Table 4). Total SOC declined by 55% (32.0 Mg ha<sup>-1</sup>) at Wushwush and by 63% (40.3 Mg ha<sup>-1</sup>) at Munesa following forest clearing and continuous cultivation, while losses of N from the cultivated fields of the two sites amounted to 52% (2.8 Mg ha<sup>-1</sup>) and 60% (3.1 Mg ha<sup>-1</sup>), respectively, after corrections for bulk density differences were made. These values are in agreement with the results of Dalal and Mayer (1986), Sombroek et al. (1993), and Solomon et al. (2000) following clearing tropical forests and their subsequent conversion in to arable land. However, if the changes in bulk density were ignored, only 42% (24.4 Mg ha<sup>-1</sup>) and 38% (24.2 Mg ha<sup>-1</sup>) depletion in total SOC and 39% (2.1 Mg ha<sup>-1</sup>) and 33% (1.7 Mg ha<sup>-1</sup>) reduction in total N were found at Wushwush and Munesa, respectively (Table 4). These results indicate that without correction for bulk density changes between the forested and cultivated fields, losses of total SOC and N could be systematically underestimated. Veldkamp (1994) arrived at a similar conclusion in his investigation conducted at the Atlantic Zone of Costa Rica. Therefore, all subsequent further calculations in this study show values with corrections for bulk density differences. The observed losses of SOC and N in the continuously cropped fields could be attributed to rapid mineralization of SOM following cultivation, which disrupts soil aggregates, and thereby increases aeration and microbial accessibility to organic matter (Guggenberger et al., 1995; Solomon et al., 2000). Moreover, reduced input of plant residues into the surface soils may also contribute to the depletion of SOM in the cultivated fields. Compared with losses of SOC, land use changes resulted in slightly lower depletion of N both at Wushwush and Munesa. Thus, the influence of management was more pronounced in SOC than in N contents. However, C/N ratios in bulk soils of the cultivated fields and the natural forests did not reveal a significant difference in SOM composition of soils under the two land use systems.

Analysis of particle-size separates showed that a large proportion of SOM was associated with clay, comprising 40 to 63% of the total SOC and 56 to 71% of soil N. The silt fraction contained 31 to 52% of SOC, and 26 to 39% of soil N. The organic matter associated with

**Table 5. Soil organic carbon (SOC) and N concentrations and C/N ratios of particle-size separates at the Wushwush and Munesa sites.**

Separate	Land Use	SOC		N		SOC	N	C/N
		g kg <sup>-1</sup> fraction	g kg <sup>-1</sup> soil	%	%			
<b>Wushwush</b>								
Sand	Natural forest	104.4a <sup>†</sup>	6.51a	10.7a	0.67a	13.0	9.0	16.1
	Cultivation	34.0b	1.81b	2.4b	0.13b	6.0	3.0	18.8
Silt	Natural forest	94.1a	7.25a	30.8a	2.37a	38.0	31.0	13.0
	Cultivation	34.3b	2.81b	11.6b	0.94b	31.0	26.0	12.2
Clay	Natural forest	70.4a	7.98a	40.2a	4.55a	49.0	60.0	8.9
	Cultivation	39.9b	4.44b	23.7b	2.63b	63.0	71.0	9.0
<b>Munesa</b>								
Sand	Natural forest	62.3a <sup>†</sup>	2.75a	8.1a	0.36a	8.0	5.0	22.6
	Cultivation	10.4b	0.38b	1.7b	0.09b	4.0	3.0	19.6
Silt	Natural forest	150.3a	9.06a	52.1a	3.14a	52.0	39.0	16.6
	Cultivation	64.3b	4.29b	18.3b	1.22b	43.0	36.0	15.0
Clay	Natural forest	76.6a	8.59a	40.1a	4.49a	40.0	56.0	8.9
	Cultivation	46.0b	4.32b	22.4b	2.10b	53.0	61.0	10.7

<sup>†</sup> Different letters along the column indicate significant differences between mean SOC and N values of the different land use systems at  $P < 0.05$  ( $n = 3$ ).

the sand fraction contained only 4 to 13% of the total SOC and 3 to 9% of soil N (Table 5). The distribution of SOC and N in size separates in these soils is similar to patterns observed in many of temperate (Amelung et al., 1998) and tropical soils (Guggenberger et al., 1995; Koutika et al., 2000; Solomon et al., 2000). The C/N ratios of the size separates decrease with decreasing diameter, suggesting that microbial alteration of SOM in the finer size separates was much higher than in the coarser size separates (Zech et al., 1997). The C/N ratio of clay fraction was lower than that of the bulk soils, while the C/N ratios of silt and sand fractions were higher than the values of the bulk soils, similar to our results from northern Tanzania (Solomon et al., 2000), indicating that the clay fraction represents the largest pool of most humified SOM in these tropical soils.

### $\delta^{13}\text{C}$ Signature of Plant and Litter Samples

Terrestrial plant tissues, fall into two major groups ( $C_3$  and  $C_4$ ) with distinct  $\delta^{13}\text{C}$  values. The average  $\delta^{13}\text{C}$  values of fresh leaves of the forest vegetation at the Wushwush and Munesa sites were  $-31.7\text{‰}$  and  $-27.7\text{‰}$ , respectively, indicating  $\delta^{13}\text{C}$  values characteristic of  $C_3$  vegetation (Table 6). More positive  $\delta^{13}\text{C}$  values were obtained from root samples of the forest vegetation ( $-28.4$  and  $-25.4\text{‰}$ ) of both sites. The  $\delta^{13}\text{C}$  values of the litter samples were intermediate between the values of the corresponding fresh leaves and roots. These results confirm that the SOC inputs in the natural forest sites were exclusively of  $C_3$  origin. Similar to the forest vegetation, the fresh maize leaves ( $C_4$ ) had slightly lower average  $\delta^{13}\text{C}$  values ( $-12.5\text{‰}$ ) than the roots ( $-12.0\text{‰}$ ) (Table 6). The  $\delta^{13}\text{C}$  values of the maize plant parts from the Wushwush and Munesa sites were similar to those reported by other researchers (Balesdent and Mariotti, 1987; Balesdent et al., 1990; Collins et al., 1999).

### $\delta^{13}\text{C}$ Signature of Bulk Soils and Particle-Size Separates

The average  $\delta^{13}\text{C}$  values of SOM in bulk soils of the natural forests ranged from  $-24.3$  to  $-23.4\text{‰}$  at Wush-

**Table 6.  $\delta^{13}\text{C}$  values (‰) of fresh leaves, roots and litter samples.**

SOC source	$\delta^{13}\text{C}$	
	Natural forest ( $C_3$ )	Cultivation ( $C_4$ )
<b>Wushwush</b>		
Fresh leaves	$-31.7 \pm 0.4$ <sup>†</sup>	$-12.5 \pm 0.3$
Roots	$-28.4 \pm 0.5$	$-12.0 \pm 0.3$
Slightly decomposed litter	$-28.8 \pm 0.2$	n.d. <sup>‡</sup>
Largely decomposed litter	$-28.7 \pm 0.2$	n.d.
Mean <sup>§</sup>	$-30.1 \pm 1.6$	$-12.2 \pm 0.3$
<b>Munesa</b>		
Fresh leaves	$-27.6 \pm 0.4$	$-12.4 \pm 0.3$
Roots	$-25.4 \pm 0.3$	$-11.9 \pm 0.2$
Litter	$-26.7 \pm 0.4$	n.d.
Mean <sup>§</sup>	$-26.5 \pm 0.9$	$-12.2 \pm 0.4$

<sup>†</sup> SD calculated for three replicates.

<sup>‡</sup> n.d., not determined.

<sup>§</sup> Mean values of fresh leaves and roots.

wush and Munesa, respectively (Fig. 1). These values were generally higher than average  $\delta^{13}\text{C}$  values of the associated fresh leaves and roots ( $-30.1$  and  $-26.5\text{‰}$ ) (Table 6). Balesdent et al. (1990) stated that in well-aerated soils which have been under  $C_3$  vegetation, SOM exhibits higher  $\delta^{13}\text{C}$  values than the original plant materials or the litter layer. This phenomenon is usually attributed to biological and biochemical transformations occurring during humification processes, which lead  $^{13}\text{C}$  enrichment of the residual organic carbon compared with the original plant material (Macko and Estep, 1984; Ågren et al., 1996). In addition, biochemical variations in plant residues might also account for the observed difference (Benner et al., 1987; Ågren et al., 1996). For example, the  $\delta^{13}\text{C}$  value of lignin is 2 to 6‰ lower than the  $\delta^{13}\text{C}$  value of the whole plant tissue (Benner et al., 1987). Hence, during the humification process, decomposition of organic structures such as lignin in the soil could promote  $^{13}\text{C}$  enrichment of SOM of the surface soils compared with the original plant materials.

Our results indicates that the  $\delta^{13}\text{C}$  values of SOM in bulk soils of the natural forests ( $-24.3$  and  $-23.4\text{‰}$ ) were significantly lower than  $\delta^{13}\text{C}$  values of soils from the cultivated fields ( $-19.9$  and  $-15.5\text{‰}$ ) at the Wushwush and Munesa sites, respectively (Fig. 1). Isotopic enrichment of SOM in surface soils of the cultivated fields is due to the maize crop ( $C_4$ ), which discriminates less against  $^{13}\text{C}$  than the original forest ( $C_3$ ) vegetation.

The  $^{13}\text{C}$  values of SOM associated with particle-size separates from forest vs. cultivated soils exhibited different patterns. In the natural forests, the  $\delta^{13}\text{C}$  value increased with decreasing particle size, the highest being observed in SOM bound to the clay ( $-23.0\text{‰}$  at Wushwush and  $-23.2\text{‰}$  at Munesa) (Fig. 1). In contrast, particle-size separates of the cultivated fields showed higher enrichment in the sand ( $-19.1$  and  $-14.5\text{‰}$ ) than in the silt ( $-21.0$  and  $-15.5\text{‰}$ ) and clay ( $-21.5$  and  $-18.3\text{‰}$ ) at the Wushwush and Munesa sites, respectively.

The  $\delta^{13}\text{C}$  values of SOM bound to the clay fraction of the Wushwush and Munesa forests were 7.1 and 3.3‰ higher than average  $\delta^{13}\text{C}$  values of the corresponding forest vegetation ( $-30.1$  at Wushwush and  $-26.5\text{‰}$  at Munesa). In contrast, the SOM associated with the sand fraction of soils from the natural forests had the lowest  $\delta^{13}\text{C}$  values ( $-26.1$ , Wushwush and  $-25.2\text{‰}$ , Munesa)

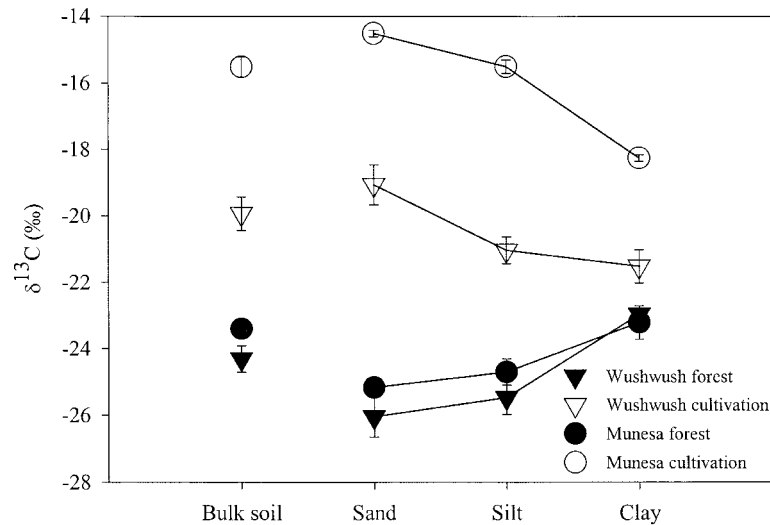


Fig. 1. Average  $\delta^{13}\text{C}$  values of bulk soil and particle-size separates in the subhumid highlands of southern Ethiopia. Each point is the mean of three replicates, and error bars represent  $\pm 1$  standard deviation.

and generally were only 4.0 and 1.3‰ higher than mean  $\delta^{13}\text{C}$  values of the original forest vegetation at the two sites, respectively. Similar patterns were observed by Desjardins et al. (1994) and Shang and Tiessen (2000) for tropical soils from Brazil and by Boutton et al. (1998) for soils collected from a subtropical savanna ecosystem in North America. The  $\delta^{13}\text{C}$  values of SOM associated with the silt fraction of both land use systems were intermediate between those of the sand and clay fractions. The mechanisms involved for the observed enrichment of  $^{13}\text{C}$  with decreasing particle size in the forest soils are not fully understood yet. However, they might be attributed to the increasing degree of humification of the associated SOM, which could involve (i) the preferential loss of  $^{12}\text{C}$  during decomposition of organic carbon, (ii) the increasing contents of  $^{13}\text{C}$  enriched organic components such as microbial tissues (Macko and Estep, 1984), which are less available to for microbial digestion (Roscoe et al., 2000; Gerzabek et al., 2001), and (iii) the decreasing contents of  $^{13}\text{C}$  depleted organic substrates such as lignin (Benner et al., 1987) in the finer size separates.

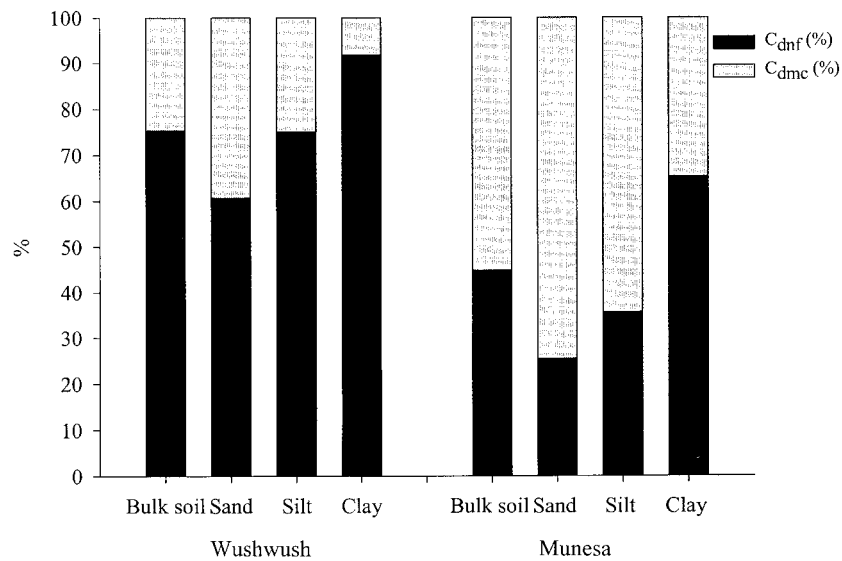
Comparison of the two land use systems at both sites indicated that the SOM associated with particle-size separates of the cultivated soils was generally more enriched in  $^{13}\text{C}$  than the corresponding size separates of the natural forest soils. This was demonstrated by the difference in  $^{13}\text{C}$  values of the corresponding size separates of the two land use systems [i.e., ( $D_d = ^{13}\text{C}$  value of sand from the cultivated fields -  $\delta^{13}\text{C}$  value of sand from the natural forest)]. According to Fig. 1, the differences in  $^{13}\text{C}$  values of similar size separates of the cultivated and forested soils were greatest in the sand separate of both sites (7.0 and 10.5‰) and decreased with decreasing particle size (silt, 4.4 and 9.2‰ and clay, 1.5 and 5.0‰) at the two sites, respectively. These results indicated that  $\delta^{13}\text{C}$  signature of organic matter input from the current vegetation ( $\text{C}_4$ ) was clearly reflected in the sand followed by the silt fraction, following a

management-inflicted vegetation change from  $\text{C}_3$  forest to  $\text{C}_4$  cropland. According to our data, however, the  $\delta^{13}\text{C}$  signatures of the original plant community ( $\text{C}_3$  forest) were more evident in the clay fraction of the cultivated soils and only little  $\text{C}$  from the newly introduced  $\text{C}_4$  vegetation reached the clay fractions during the decomposition process of SOM in these tropical agroecosystems.

#### Percentages and Amounts of SOC Derived from Maize and Forest Vegetation

The effects of  $\text{C}_3$  to  $\text{C}_4$  vegetation change were reflected by estimates of the amount of SOC derived from maize ( $\text{C}_{\text{dmc}}$ ) and forest ( $\text{C}_{\text{dnf}}$ ) residues in surface soils (0–10 cm) of the two cultivated fields. After 25 yr of continuous cultivation at the Wushwush site, on average only 25% (6.5  $\text{Mg ha}^{-1}$ ) of the existing total SOC was derived from maize. The remaining 75% (19.8  $\text{Mg ha}^{-1}$ ) of the total SOC in the cultivated soils was still attributed to the natural forest vegetation (Fig. 2 and Table 2). However, at the Munesa site, after 30 yr of continuous maize cropping, 55% (13.1  $\text{Mg ha}^{-1}$ ) of the existing total SOC was derived from maize, while only 45% (10.6  $\text{Mg ha}^{-1}$ ) of the total SOC in bulk soils of the cultivated fields originated from the preceding  $\text{C}_3$  forest vegetation.

The average proportions of forest-derived SOC lost as a result of 25 and 30 yr of continuous cropping amounted to 66% (38.5  $\text{Mg ha}^{-1}$ ) at the Wushwush and 83% (53.3  $\text{Mg ha}^{-1}$ ) at Munesa (Fig. 3), which was similar to results for managed soils from the savannas of Ivory Coast (Martin et al., 1990) and to the humid tropical soils of Brazil (Desjardins et al., 1994). The strong changes in isotope signature of bulk soils from the two land use systems showed that clear cutting and continuous cultivation resulted in a marked depletion of the original forest-derived SOC in surface soils of these subhumid tropical agroecosystems. Our results also indicated that the losses of initial forest-derived



**Fig. 2.** Proportion of forest- ( $C_{dnf}$ ) and maize-derived ( $C_{dmc}$ ) SOC in bulk soils and particle-size separates of two cultivated soils in the southern Ethiopian highlands.

SOC were not fully compensated by the SOC input from maize crop residues in these subhumid tropical agroecosystems. The observed net losses of SOC and slower SOC accretions in the cultivated fields were likely due to accelerated decomposition of SOM following tillage coupled with the reduction of litter input and removal of crop residues from the cultivated fields. According to Fig. 3, the relative depletion of forest-derived SOC after forest clearing and subsequent cropping was higher in the surface soil of the Munesa than that of the Wushwush site. This difference may be explained by the difference in duration of cultivation, local climatic variations and vegetation type, or by the difference in land preparation, which involves burning the initial forest-derived organic matter after clear cutting of the existing natural forest at the Munesa.

The relative proportion of SOC derived from forest vs. maize varied strongly within the size separates. The proportion of SOC derived from forest vegetation of both sites increased progressively in the following order: sand < silt < clay (Fig. 2). After 25 and 30 yr of continuous cultivation, 92% ( $21.7 \text{ g kg}^{-1}$  soil) and 65% ( $14.6 \text{ g kg}^{-1}$  soil) of the SOC present in the clay fraction still exhibits a forest signature, respectively (Fig. 2 and Table 3). The proportion of SOC derived from maize showed the opposite pattern. The highest percentage of SOC derived from maize crop residue was found in the sand (39%,  $0.9 \text{ g kg}^{-1}$  soil at Wushwush and 75%,  $1.3 \text{ g kg}^{-1}$  soil at Munesa), followed by the silt and clay fractions. These findings further indicate that organic matter input from maize crop residue was more evident in the sand, followed by the silt fraction. However, the majority of SOM associated with clay was derived from the original  $C_3$  forest vegetation and that relatively little organic matter from the  $C_4$  crop has been incorporated into clay fraction of these tropical soils. These results are consistent with other studies demonstrating the slow turnover rate of clay associated SOM, which might be

attributed to its intrinsic resistance against microbial attack and to the interactions with the mineral constituents and entrapment within clay aggregates (Balesdent et al., 1988; Martin et al., 1990; Bonde et al., 1992).

Examination of size separates indicated that rapid decline of SOC derived from the native forest vegetation occurred in size separates of the cultivated soils. Twenty-five and 30 yr of continuous cultivation at the Wushwush and Munesa sites resulted in depletion of 80 and 96% of the initial forest-derived SOC associated with the sand fraction, while 73 and 85% of the forest-derived SOC was lost from the silt separates of the two sites, respectively (Fig. 3). Forest-derived SOC in the clay separates declined only by 48 and 61% at Wushwush and Munesa for the same period of cultivation, suggesting that the clay fraction retained SOC derived from the previous vegetation more efficiently than did the silt and sand fractions.

Natural  $^{13}\text{C}$  abundance technique clearly showed that in these tropical soils, individual size separates represent SOM pools that have different turnover rates. As already observed in other temperate (Christensen, 1987) and tropical soils (Vitarello et al., 1989; Bonde et al., 1992), a rapid turnover and substitution of SOC derived from forest vegetation by SOC derived from maize crop residue was observed in the macroorganic matter associated with sand fraction. Thus, the SOC in the sand fraction was a very labile component of the SOM and is a more sensitive indicator of changes in soil carbon storage in response to changes in agricultural management practices. However, a substantial amount of forest-derived SOC was also lost from the silt fraction, suggesting that SOM associated with the silt was also susceptible to land use changes and, at least in the soils under study, represents a moderately labile SOM pool, which is generally not the case in temperate soils (Christensen, 1987). The lower depletion of forest-derived SOC from clay fractions indicates that the SOM bound to the clay



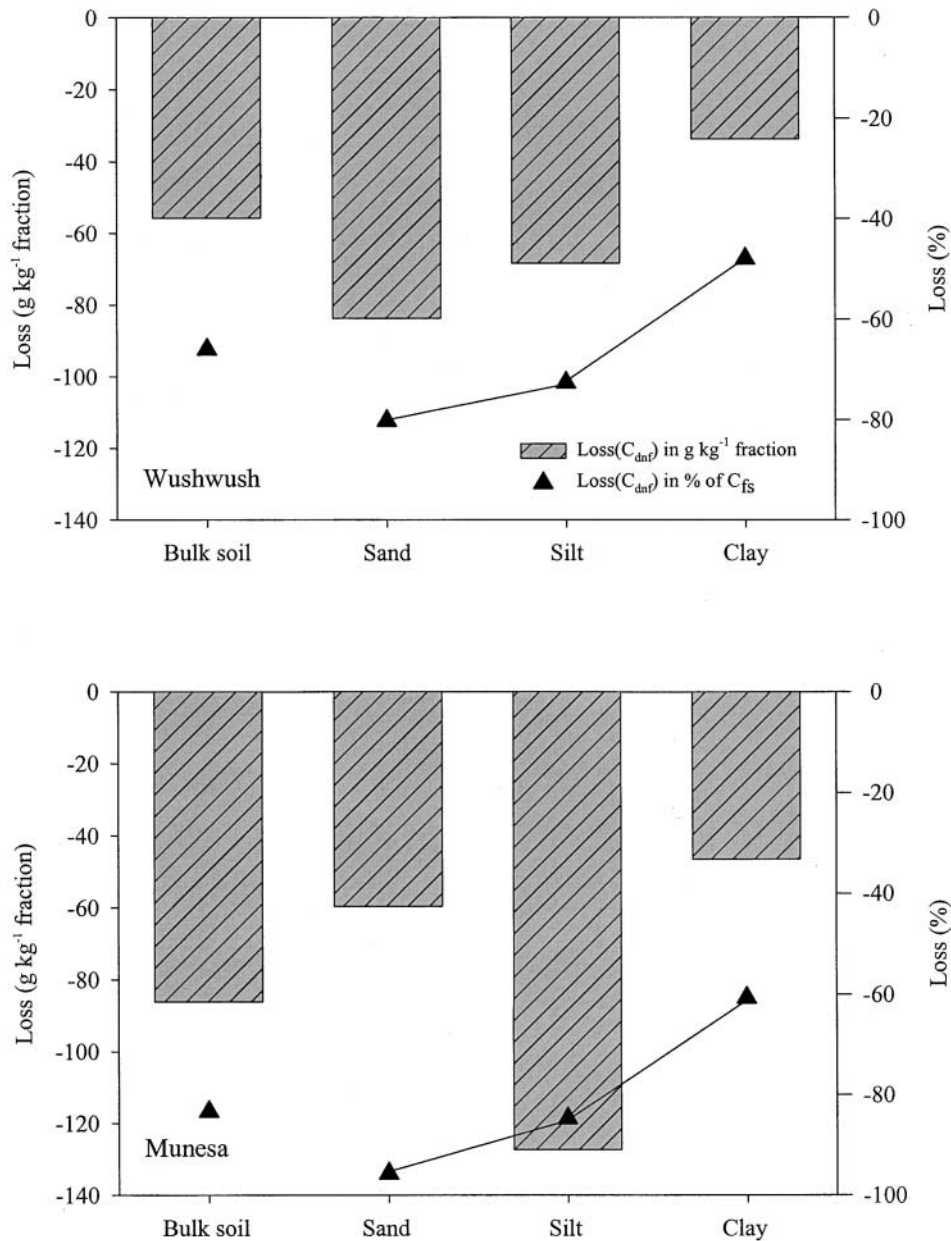


Fig. 3. Loss of forest-derived SOC ( $\text{Loss}(C_{\text{dnf}})$ ) in bulk soil and particle-size separates of cultivated fields at the Wushwush and Munesa sites.

was more stable than that found in the sand and silt fractions of the studied soils. The relative stability of clay-bound SOM in these tropical soils could be attributed to physical and chemical stabilization of SOM against biodegradation by clay minerals and clay associated sesquioxides (Zech et al., 1997).

### CONCLUSIONS

Natural  $^{13}\text{C}$  abundance technique coupled with particle-size fractionation clearly demonstrated that the soil ecosystems of the southern Ethiopian highlands are highly sensitive to changes in vegetation cover caused by land use changes. Deforestation followed by continuous maize cropping resulted in significant reduction of forest-derived SOC ( $C_{\text{dnf}}$ ) in bulk soils and in all size sepa-

rates (sand, silt, and clay) in these subhumid tropical highland agroecosystems. Moreover, losses of forest-derived SOC were not fully compensated by SOC input from the maize crop residues ( $C_{\text{dmc}}$ ). It is not clear if present SOC stocks have achieved a new equilibrium, or if loss may continue in these maize-cropping systems. Net losses of SOC and slower C accretions from maize cropping systems are likely due to the effects of tillage coupled with reduced organic matter inputs and removal of residues from cultivated fields. Stable C isotopes clearly indicated that, in these tropical soils, individual size separates encompass SOM pools that differ in turnover rates. As already observed in other temperate and tropical soils, turnover and substitution of SOC derived from the natural forest vegetation ( $C_{\text{dnf}}$ ) by SOC derived



from maize crop residue ( $C_{\text{dmc}}$ ) occurred more rapidly in the macroorganic matter associated with sand than in SOM associated with the silt and clay fractions. Thus, the SOC in the sand fraction was a very labile component of the SOM and is a more sensitive indicator of changes in soil carbon storage in response to land use changes. However, substantial amount of forest-derived SOC was also lost from the silt fraction, suggesting that SOM associated with the silt was also susceptible to management changes and that, at least in the soils under study, represents a moderately labile SOM pool, which is generally not the case in temperate soils. The lower depletion of forest-derived SOC from clay fractions indicates that the SOM bound to the clay was more stable than that found in the sand and silt fractions, indicating the importance of organomineral associations in stabilization of SOM in these tropical soils.

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