

See discussions, stats, and author profiles for this publication at: <http://www.researchgate.net/publication/228644004>

Influence of land use on the humic substances of some tropical soils of Nigeria. Eur J Soil Sci

ARTICLE *in* EUROPEAN JOURNAL OF SOIL SCIENCE · JUNE 2005

Impact Factor: 2.65 · DOI: 10.1111/j.1365-2389.2004.00671.x

CITATIONS

23

READS

41

4 AUTHORS, INCLUDING:



[Alessandro Piccolo](#)

University of Naples Federico II

306 PUBLICATIONS 6,349 CITATIONS

SEE PROFILE



[Pellegrino Conte](#)

Università degli Studi di Palermo

125 PUBLICATIONS 2,389 CITATIONS

SEE PROFILE



[Riccardo Spaccini](#)

University of Naples Federico II

91 PUBLICATIONS 1,764 CITATIONS

SEE PROFILE

Influence of land use on the characteristics of humic substances in some tropical soils of Nigeria

A. PICCOLO^a, P. CONTE^a, R. SPACCINI^a & J. S. C. MBAGWU^b

^a*Dipartimento di Scienze del Suolo, delle Piante e dell' Ambiente, Università di Napoli Federico II, Via Università 100, 80055 Portici, Napoli, Italy, and* ^b*Department of Soil Science, University of Nigeria, Nsukka, Nigeria*

Summary

In highly weathered tropical conditions, soil organic matter is important for soil quality and productivity. We evaluated the effects of deforestation and subsequent arable cropping on the qualitative and quantitative transformation of the humic pool of the soil at three locations in Nigeria. Cultivation reduced the humic pool in the order: acetone-soluble hydrophobic fraction (HE) > humic acid (HA) > humin (HU) > fulvic acid (FA), but not to the same degree at all three sites. The C and N contents, as well as the C/N ratios of humic extracts, were large and not substantially influenced by land use. The $\delta^{13}\text{C}$ values of the humic extracts were invariably more negative in forested soils thereby showing a dilution of $\delta^{13}\text{C}$ signature with cultivation from C3 to C4 plants. The $\delta^{13}\text{C}$ values of apolar HE fractions were generally more negative, indicating a reduced sensitivity compared with other humic fractions to turnover of crop residues. The contents of hydrophobic constituents (alkyl and aromatic C), as revealed by cross-polarization magic angle spinning (CPMAS) ^{13}C -NMR spectroscopy, in HA, FA and HU were generally < 50%, with the exception of larger hydrophobicity in HU in the forested soil at Nsukka and HA in that at Umudike. The HE fraction contained significantly more apolar constituents, and consequently had a larger intrinsic hydrophobicity than the other humic fractions. The larger reduction of apolar humic constituents than of the less hydrophobic humic fractions, when these soils were deforested for cultivation, indicates that at those sites the stability of accumulated organic matter is to be ascribed mainly to the selective preservation of hydrophobic compounds.

Introduction

In the tropics, soil organic matter determines the fertility and productivity of soils, especially when these are highly weathered, with small or no reserves of nutrients, and are managed without any external inputs of organic or inorganic fertilizer (Feller & Beare, 1997; Zech *et al.*, 1997). Moreover, at the global scale the type of land use will affect the capacity of the soil to act as both a source and a sink of organic matter, nutrients and atmospheric CO_2 . It is therefore essential that the influence of land use on the dynamics of soil organic matter in the tropics should be more precisely clarified.

Massive deforestation in the tropics has resulted in loss of soil productivity through reduction in organic C content and consequent loss in aggregate stability (Alegre & Cassel, 1986). Since the aggregates and particle-size fractions are the seat of organic matter, their loss through rapid decomposition often accompanies deforestation (Piccolo, 1996). Moreover, higher

temperatures and moisture regimes also enhance soil organic C turnover rates in tropical soils as compared with temperate ones (Ayanaba & Jenkinson, 1990), thereby increasing the potential risk of soil degradation. Several studies have focused on organic matter dynamics in tropical soils of South America (Desjardins *et al.*, 1994) and a few in soils of the West African Sahelian region (Dutartre *et al.*, 1993). Some of the studies have dealt with carbon storage as influenced by land use (Lepsch *et al.*, 1994) or the estimate of organic matter turnover using the ^{13}C natural abundance technique (Martin *et al.*, 1990).

Soil organic matter is composed of different compartments which differ from each other in biochemical composition, biological stability and carbon turnover rates. The humified organic matter, namely humic acid (HA), fulvic acid (FA) and humin (HU), is supposed to represent the most microbially recalcitrant and thus stable reservoir of organic carbon (OC) in soil and is an important component for the control of soil erosion by water and wind (Piccolo, 1996). Most of the studies on organic matter dynamics in the tropics have focused on its absolute amount without recourse to the chemical fractions.

Correspondence: A. Piccolo. E-mail: alpiccol@unina.it

Received 22 November 2001; revised version accepted 24 June 2004

Zech *et al.* (1997) reviewed several studies that have utilized cross-polarization magic angle spinning (CPMAS) ^{13}C -NMR to characterize the humification process of soil organic matter (SOM) in the tropics. In the highly weathered, lowland, humid, tropical soils of Nigeria and similar soils, there is no information on the changes of the stable humic pool following deforestation and consequent arable farming. Yet more than 70% of the cation exchange capacity (CEC) of these soils is attributed to SOM and to HA in particular (Asadu *et al.*, 1997).

In this study, we evaluated the qualitative and quantitative changes associated with the composition of humic substances following the conversion of tropical forests at three sites in Nigeria to arable farming. The aim of this study was to contribute to the knowledge of organic matter dynamics in a humid region of Africa.

Materials and methods

Soil sampling and preparation

Geographical and agronomic information on the three locations in Nigeria were given in detail by Spaccini *et al.* (2001), whereas clay mineralogy, soil classification, and some of the soil physico-chemical properties for each location are given in Table 1. The clay fraction was obtained by slow sedimentation in water after destruction of SOM with Na-hypochlorite. The X-ray diffractograms were obtained on oriented clay samples

saturated with glycerol or MgCl_2 , using a Siemens D500 diffractometer with $\text{Cu-K}\alpha$ radiation.

The experimental plots belong to the University of Nigeria and had been deforested mechanically in the 1970s followed up by hand-hoeing. Small forest plots were conserved nearby (< 1 km) and consisted of local rainforest species. The cultivated sites for this study have run continuously since 1990 (Abakiliki and Umudike) and 1991 (Nsukka) under arable cropping without inputs of organic fertilizers. At each site the experiment was set up as a randomized complete block design with three replicates of each of the forested (F) and cultivated (C) treatments. Each plot size was $6\text{ m} \times 5\text{ m}$. At Abakiliki and Nsukka, cultivation consisted essentially of hand-hoeing and planting without weed control, fertilizer addition and mechanical tillage. At Umudike mechanical cultivation (disking, ploughing and harrowing at about 20 cm depth) was used. In September 1995, three bulked topsoil (0–20 cm) samples, collected from each replicate of the treatments, were air-dried, sieved through a 4.75-mm aperture mesh and shipped to Italy for fractionation of humic substances and their characterization.

The < 2 mm fractions were used to determine particle-size distributions (after complete dispersion with sodium hexametaphosphate) by the pipette method of Gee & Bauder (1986). The soil pH was measured in 1:2.5 soil/water ratio, OC by the wet oxidation method of Nelson & Sommers (1982) and converted to OM by multiplying by 1.724, and

Table 1 Sampling site, soil classification, and some properties of soils (standard deviations in parentheses)

Site	Soil order	Texture ^a	Clay minerals ^b	pH (H_2O)	Organic matter /g kg ⁻¹	CEC ^c /cmol _c kg ⁻¹	$\delta^{13}\text{C}$ /‰
Abakiliki 06°30'N, 08°15'E	Inceptisol	LS	Mi ^A , K ^B , Sm ^C	Forested			
				5.59	41.4	34.5	-26.02
				(0.32)	(6.4)	(1.8)	(0.11)
				Cultivated			
5.54	17.9	23.0	-21.18				
(0.27)	(0.9)	(3.3)	(0.12)				
Nsukka 06°51'N, 07°24'E	Ultisol	SCI	K ^A	Forested			
				3.53	20.7	15.0	-28.17
				(0.16)	(0.9)	(3.6)	(0.14)
				Cultivated			
3.87	11.7	14.5	-19.22				
(0.21)	(2.1)	(0.7)	(0.13)				
Umudike 05°29'N, 07°33'E	Ultisol	SCI	K ^A	Forested			
				4.21	4.1	24.0	-27.28
				(0.28)	(0.5)	(1.1)	(0.16)
				Cultivated			
4.53	1.0	11.0	-22.16				
(0.14)	(0.3)	(0.8)	(0.12)				

^aLS, loamy sand; SCI, sandy clay loam.

^bMi, mica; Sm, smectite; K, kaolinite; A = abundant; B = present; C = few.

^cCEC, cation exchange capacity.

NH₄OAc-exchangeable bases and CEC by the procedures described by Thomas (1982).

Extraction of humic substances

The specific humic fractions were isolated (Piccolo *et al.*, 1998) from the surface horizon (0–20 cm) of each replicate soil from forested and cultivated treatments. The following fractions were obtained:

1 Humic (HA) and fulvic acids (FA) were extracted with a combined 1 M NaOH and 1 M Na₄P₂O₇ solution (50:50, v:v).

2 A so-called humin (HU) fraction was extracted from the soil residue remaining after the NaOH–Na₄P₂O₇ extraction of HA and FA. The soil residue was first washed with distilled water until neutrality of washings and then shaken twice with a 10% HF solution for 24 hours. After centrifugation at 1510 g for 30 minutes, the supernatant was discarded while the residue was washed to neutrality with distilled water and again extracted with a 1 M NaOH–Na₄P₂O₇ solution. This extract (HU) was subsequently treated as for HA.

3 A humic extract (HE) was solubilized in a solution of acetone–0.6 M HCl (8:2, v:v). Briefly, soil samples were placed in rubber-stoppered polyethylene bottles with the extracting solution (1:5, w:v) and shaken for 24 hours on a mechanical shaker. The mixture was centrifuged, and the supernatant containing the extract (HE) was filtered through a glass microfibre filter (Whatman GF/C, 47 mm). The soil residue was further extracted twice with the same extracting solution. The HE from subsequent extractions was combined, rotoevaporated and dialysed against water to eliminate the solvent completely.

For the alkaline extractions, the suspensions holding HA and FA, filtered once through glass wool, were combined and brought rapidly to pH 1 with concentrated HCl, and the HA was allowed to precipitate for 24 hours at 20°C. The precipitated HA was separated 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 passing through non-ionic polymeric resins (XAD-8, SERVA Chemicals) to remove the non-humified organic compounds (mainly saccharides). The eluted FA were brought to pH 5 with HCl, dialysed 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 hours with 0.5% HCl–HF solution until the ash content was <5%. The humic fractions were then dialysed against water until free of Cl[–], freeze-dried and stored for further analysis.

NMR spectroscopy

The ¹³C-NMR spectra of humic extracts from the soils were recorded by the CPMAS technique. The CPMAS NMR spectra were obtained for every replicate extract of every sample. 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 in the evaluation of peak areas (Conte *et al.*, 1997). The OCT ranged between 0.8 and 1.0 ms. The line broadening for free induction decay transformation was fixed at 50 Hz. The following resonance intervals were associated with the different carbons (Preston & Newman, 1995): alkyl C: 0–50 p.p.m.; C-N in polypeptidic compounds: 50–85 p.p.m.; C-O in carbohydrate compounds: 85–105 p.p.m.; aromatic C: 105–160 p.p.m.; carboxylic-C: 160–200 p.p.m. The areas relative to these resonance intervals (p.p.m.) were used to evaluate the degree of hydrophobicity (HB/HI) of the humic fractions:

$$\text{HB/HI} = [(0-50) + (105-160)] / [(50-105) + (160-200)]. \quad (1)$$

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 (Piccolo & Conte, 2003).

Elemental and δ¹³C content of humic fractions

The isotopic abundance (δ¹³C) 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), and results expressed as ‰, as the deviation of the isotopic ratio of the sample from that of an arbitrary standard:

$$\delta^{13}\text{C} \text{ ‰} = [(R_{\text{sample}} - R_{\text{standard}}) / R_{\text{standard}}] \times 1000, \quad (2)$$

where R_{sample} is ¹³C/¹²C of the sample and R_{standard} is ¹³C/¹²C of the Pee Dee Belemnite (PDB) standard (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.

Results and discussion

Yields of humic fractions

The extraction yields 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 decrease in SOM (Table 1), a progressive reduction in these humic fractions was found for all soils as forests were converted to arable farming. The magnitude of reduction was, however, site-specific. More than 80% of the reduction in HE extracted with the acetone–HCl mixture occurred at Abakiliki and Nsukka whereas the smallest reduction was obtained at Umudike (60.9%). Abakiliki and Nsukka had a greater reduction in

Table 2 Yields of extraction (g kg⁻¹ of soil) and relative standard deviations (in parentheses) for humic acids (HA), fulvic acids (FA), humin (HU) and humic extract (HE)^a from forested (F) and cultivated (C) soils in Nigeria

Site	HA	FA	HU	HE
<i>Abakiliki</i>				
F	12.9 (1.2)	7.8 (1.1)	3.4 (0.4)	3.0 (0.2)
C	5.6 (0.4)	3.9 (0.3)	1.2 (0.2)	0.6 (0.2)
<i>Nsukka</i>				
F	5.4 (1.2)	4.1 (1.3)	1.0 (0.2)	2.7 (0.2)
C	2.0 (0.9)	1.9 (0.6)	2.0 (1.1)	0.4 (0.1)
<i>Umudike</i>				
F	5.2 (0.4)	2.5 (0.2)	2.4 (0.6)	2.3 (0.3)
C	3.9 (0.9)	1.6 (0.5)	1.3 (0.3)	0.9 (0.3)

^aExtracted by acetone-HCl solution.

HA (56.6 and 55.5%, respectively) and FA (50.0 and 53.7%, respectively) than Umudike (25.0% for HA and 36.0% for FA). The reduction in HU at Abakiliki and Umudike (64.7 and 45.8%, respectively) was significant, according to standard deviation, whereas the increase in HU (50%) passing from forest to cultivation at Nsukka was not significant (Table 2).

Similar reductions in these humic fractions have been reported in other studies (Spaccini *et al.*, 2001). Reduction of OM with progressive cultivation is commonly attributed to microbial oxidation of the partially decomposed organic materials or clay-humic complexes which were previously protected in soil aggregates and became disrupted as a result of farming practices (Piccolo, 1996). These findings indicate that decomposition affected the soil content in HE and HA more than in other humic fractions after soils were cultivated.

Carbon, nitrogen and isotopic $\delta^{13}\text{C}$ content of humic fractions

The C and N contents of the humic extracts and the relative C/N ratios are reported in Table 3. Their values are within the ranges found for other soils (Piccolo *et al.*, 1998). At Abakiliki, the C content decreased significantly in the HA and HE fractions, passing from the forested to the cultivated soils, whereas HU remained substantially unchanged and FA showed a slight increase. The C content in the humic fractions of Nsukka revealed a similar behaviour, though to a lesser extent. Conversely, at Umudike, it was the FA fraction that showed a significant decline in C content as a result of cultivation, whereas the rest of the humic fractions did not change substantially. The N content of the HA and HE fractions decreased from forested to cultivated soils at all sites, while the HU fraction remained substantially unchanged. The FA fraction showed contrasting results since its N content decreased at Nsukka and Umudike but increased significantly at Abakiliki, when passing from forested to cultivated sites.

Table 3 C and N content (%) and $\delta^{13}\text{C}$ values (‰) of humic fractions (HA, FA, HU, HE) from forested (F) and cultivated (C) sites of Nigeria (standard deviations in parentheses)

Fraction	Site	C /%	N /%	C/N ratio	$\delta^{13}\text{C}$ /‰
<i>Abakiliki</i>					
HA	F	56.64 (0.15)	3.12 (0.03)	18.15 (3.27)	-25.97 (0.14)
	C	50.71 (0.16)	2.63 (0.05)	19.28 (4.05)	-21.13 (0.16)
FA	F	36.52 (0.06)	1.77 (0.05)	20.63 (2.27)	-26.36 (0.23)
	C	38.03 (0.22)	2.95 (0.03)	12.89 (3.22)	-20.97 (0.14)
HU	F	54.42 (0.21)	3.32 (0.06)	16.39 (4.43)	-24.45 (0.11)
	C	54.31 (0.15)	3.32 (0.04)	16.35 (3.11)	-20.42 (0.15)
HE	F	62.44 (0.13)	1.71 (0.03)	36.51 (5.84)	-26.81 (0.20)
	C	56.90 (0.13)	1.61 (0.02)	35.34 (5.30)	-23.27 (0.14)
<i>Nsukka</i>					
HA	F	54.29 (0.17)	4.30 (0.04)	12.56 (2.65)	-27.31 (0.09)
	C	53.12 (0.20)	3.18 (0.05)	16.70 (3.34)	-17.22 (0.12)
FA	F	36.35 (0.11)	2.68 (0.03)	13.56 (1.90)	-26.89 (0.18)
	C	38.93 (0.13)	2.35 (0.02)	16.56 (2.48)	-17.25 (0.16)
HU	F	49.77 (0.13)	3.10 (0.06)	16.05 (3.05)	-27.79 (0.13)
	C	49.57 (0.17)	3.02 (0.04)	16.41 (3.45)	-17.17 (0.10)
HE	F	61.67 (0.12)	2.48 (0.03)	24.86 (3.73)	-27.78 (0.19)
	C	60.15 (0.16)	2.33 (0.03)	25.81 (4.90)	-17.97 (0.18)
<i>Umudike</i>					
HA	F	55.32 (0.12)	3.87 (0.03)	14.29 (2.14)	-27.10 (0.12)
	C	55.41 (0.14)	2.90 (0.02)	19.01 (3.06)	-23.31 (0.16)
FA	F	39.66 (0.14)	2.91 (0.02)	13.62 (2.18)	-26.99 (0.21)
	C	26.03 (0.15)	1.85 (0.02)	14.07 (2.39)	-22.38 (0.11)
HU	F	50.74 (0.18)	3.95 (0.05)	12.84 (2.95)	-26.53 (0.17)
	C	50.67 (0.16)	3.80 (0.03)	13.34 (2.53)	-22.99 (0.17)
HE	F	57.30 (0.20)	3.58 (0.03)	16.00 (3.68)	-26.79 (0.13)
	C	57.95 (0.19)	2.23 (0.03)	25.98 (4.99)	-24.09 (0.21)

The C/N ratios followed the changes in C and N content in the humic fractions. However, the values did not indicate significant changes for the majority of humic fractions in all soils when sites were converted from forest to cultivation. Significant exceptions were the decrease of FA at Abakiliki and the increase of HE at Umudike, reflecting the N content that increased in the former fraction and decreased in the latter. The general constancy of C/N values for the different humic fractions indicates the ability of the fractions to resist microbial decomposition because of their recalcitrant nature due to either physical protection in microaggregates (Dutartre *et al.*, 1993) or chemical protection by their hydrophobic composition (Spaccini *et al.*, 2000).

Isotopic ($\delta^{13}\text{C}$) measurements of SOC have been utilized successfully to document effects of land use changes on turnover rates of OM (Bernoux *et al.*, 1998). Such studies have been done in particle-size fractions, aggregate size classes and free particulate OM with a density of > 1.6 g cm⁻³. Organic matter in sand-sized fractions has faster turnover rates than that in clay/silt size fractions (Desjardins *et al.*, 1994), and OM turnover rates are greater in macro- than in microaggregates

(Puget *et al.*, 1995). Also, OM present in soil fractions with low densities tends to have a more rapid turnover rate than that present in soil fractions with greater densities (Gregorich *et al.*, 1995). Despite the importance of humic matter in the accumulation of OM in soils, little investigation has been conducted on the variation of $\delta^{13}\text{C}$ values in humic fractions of soils subjected to land use change (Lichtfouse *et al.*, 1998; Spaccini *et al.*, 2000) and even less in tropical soils (Zech & Guggenberger, 1996; Zech *et al.*, 1997).

The isotopic $\delta^{13}\text{C}$ values of humic extracts given in Table 3 show that with the exception of Nsukka cultivated soil, where the OC is from C4 plants, the rest are dominated by C3 plants. For the forested sites, the values are consistent with the dominant tropical vegetation, which has the C3 photosynthetic pathway, existing at these locations for more than 100 years. According to Bernoux *et al.* (1998), terrestrial plants with the C3 (Calvin cycle) pathway have $\delta^{13}\text{C}$ values in the -35 to -20% range whereas those with the C4 (Hatch–Slack) pathway have larger $\delta^{13}\text{C}$ values, in the -19 to -9% range (Boutton *et al.*, 1998). The larger (less negative) $\delta^{13}\text{C}$ values in the cultivated soils are a result of the introduction of C4 plants during cultivation. This was verified in all humic fractions of this study that showed greater $\delta^{13}\text{C}$ values when soils were converted to cultivation, and reached a peak in the HU fraction in the Nsukka cultivated soil with a $\delta^{13}\text{C}$ value of -17.17% . This value indicates the dominance of C4 plants such as grasses, which were removed before cropping with cassava (*Manihot esculenta* Crantz). About 20 years ago the dominant vegetation at this site was *Eupatorium odoratum* (a C3 plant) which decomposes very fast (Agbim, 1987), but was replaced by the present grasses dominated by *Panicum* spp. (a C4 plant). Nadelhoffer & Fry (1988) have explained that the isotopic composition of SOM is identical to 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. However, while acidic hydrolysates from the same soils did not show any incorporation of carbon from C4 plants (Spaccini *et al.*, 2004), humic materials in this study revealed a significant enrichment of ^{13}C after a relatively short period of cultivation (Table 3).

Flexor & Volkoff (1977), who studied changes in $\delta^{13}\text{C}$ values of humus chemical fractions of a humic Ultisol profile under Brazilian tropical forest, showed that the humic fraction had large negative $\delta^{13}\text{C}$ values. However, Wedin *et al.* (1995) argued that the selective preservation of specific groups of compounds (such as lignin) does not explain the pattern of $\delta^{13}\text{C}$ in all cases. In fact, we found that the HE fraction maintained significantly larger $\delta^{13}\text{C}$ values than the rest of the humic fractions in all soils converted to cultivation, thereby showing a different selective transformation in respect to other humic fractions. Spaccini *et al.* (2000) studied the changes of $\delta^{13}\text{C}$ values of HE fractions with a year-long incubation of maize residues in soils of a climosequence in Europe. They found that not only was the ^{13}C isotopic content of the

HE fraction slightly affected by decomposition of the C4 maize tissues but it exerted a chemical protection on the rest of the humic pool. They attributed this effect to the highly hydrophobic composition of the HE fraction that limits the diffusion of water and microorganisms to other humified components. The slightly more negative $\delta^{13}\text{C}$ values found for HE in both forested and cultivated sites (Table 3) thus also suggest that in these Nigerian soils this hydrophobic humic fraction has a slower turnover than the other humic fractions. This explanation is also supported by the large C/N ratios found for the HE fraction in all sites.

CPMAS ^{13}C -NMR spectra of humic extracts

The solid-state ^{13}C -NMR spectra of HA, FA and HU of the forested and cultivated soils are shown in Figure 1 whereas those of HE are reported in Figure 2. The carbon distributions in the different chemical shift (p.p.m.) intervals for the relative fractions are shown in Table 4. In spite of the quantitative error in the solid-state NMR technique, which was kept small ($<5\%$) by applying the VCT technique, the spectra of HA, FA and HU showed that the structure of humic matter was affected by deforestation and subsequent arable cropping.

At Abakiliki, the alkyl region (0–50 p.p.m.) of HA and HU increased significantly as a result of cultivation. The magnitude of the increase was 30.6% for both HA and HU, while FA remained unchanged. At Nsukka, no significant differences were observed in the alkyl content of HA and FA, while HU decreased substantially. At Umudike, a slight reduction of alkyl content in HA following cultivation was compensated by its increase in FA and HU.

Signals relative to aromatic carbon (105–160 p.p.m.) were generally largest in the forested and cultivated HU, followed by HA, and least in FA at the three sites. However, cultivation caused a slight concentration of aromatic carbon in the HA fraction for Abakiliki and Nsukka. The aromatic content of the FA fraction did not change at Abakiliki and Umudike, while it decreased significantly with cultivation at Nsukka. The aromatic content of the HU fraction showed an increase with cultivation at Abakiliki and an even more significant enhancement at Nsukka, whereas it was decreased significantly at Umudike.

At Abakiliki, the carboxyl-C signal (160–200 p.p.m.) decreased significantly by 13.4% in HA, remained substantially unchanged in HU, but increased by 38.1% in FA following cultivation. At Nsukka, the same resonance region increased for HA (14.4%), FA (32.1%) and HU (35.7%) with cultivation. Also at Umudike, the carboxyl-C increased significantly in all humic fractions as a result of cultivation (Table 4). The increase in the carboxyl-C of the majority of these soils may be attributed to the side-chain oxidation of plant-derived lignin–phenolic compounds which is likely to increase as a result of cultivation (Zech & Guggenberger, 1996) or to incorporation of carboxyl-rich material from fresh vegetal tissues

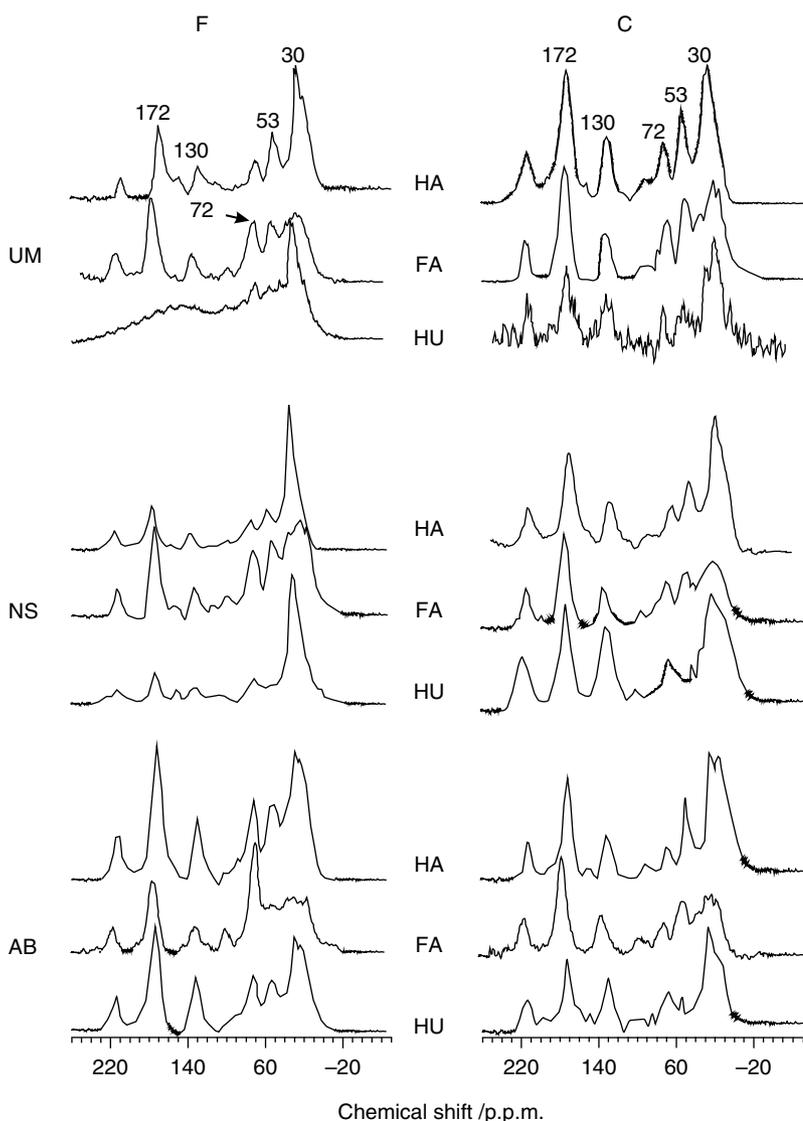


Figure 1 CPMAS ^{13}C -NMR spectra of humic acid (HA), fulvic acid (FA) and humin (HU) fractions extracted from Nigerian soils (UM, Umudike; NS, Nsukka; AB, Abakiliki) at forested (F) and cultivated (C) sites.

(Spaccini *et al.*, 2000). The peptidic-C (50–85 p.p.m.) revealed a generally significant reduction following cultivation in the HA, FA and HU fractions at all sites, thereby indicating rapid turnover of this carbon type under the new land use. The anomeric-C (85–105 p.p.m.), a signal attributed to saccharidic carbons, decreased at Umudike in all humic fractions but increased in FA at Abakiliki and in HU at Nsukka following cultivation.

These changes may be better evaluated by following the content of hydrophilic (HI) and hydrophobic (HB) carbons as well as the overall hydrophobicity (HB/HI) in the humic fractions (Table 4). The hydrophilic carbon in HA decreased due to cultivation at Abakiliki, remained unchanged at Nsukka but increased at Umudike. Concomitantly, the hydrophobic carbon in the HA fraction increased significantly at Abakiliki and showed smaller variation at Nsukka and Umudike, resulting in an HB/HI ratio for HA that increased only at

Abakiliki. The HI in FA was about the same at Abakiliki and Umudike but increased somewhat at Nsukka following cultivation, whereas that of HU decreased at Abakiliki by 16%, increased at Nsukka by 43.3%, but remained stable at Umudike. The consequence was that the degree of hydrophobicity in FA did not vary at Abakiliki and showed a decrease at Nsukka and an increase at Umudike. Conversely, the hydrophobicity of HU increased with cultivation at Abakiliki, decreased at Nsukka and remained unchanged at Umudike.

In general, when the degree of hydrophobicity was averaged between forested and cultivated soils, it became evident that the HA, FA and HU fractions followed a different dynamic pathway at Abakiliki in comparison with that at Nsukka and Umudike. A review of the factors controlling organic matter dynamics in tropical soils, carried out by Zech *et al.* (1997), indicated that changes in OM during the process of

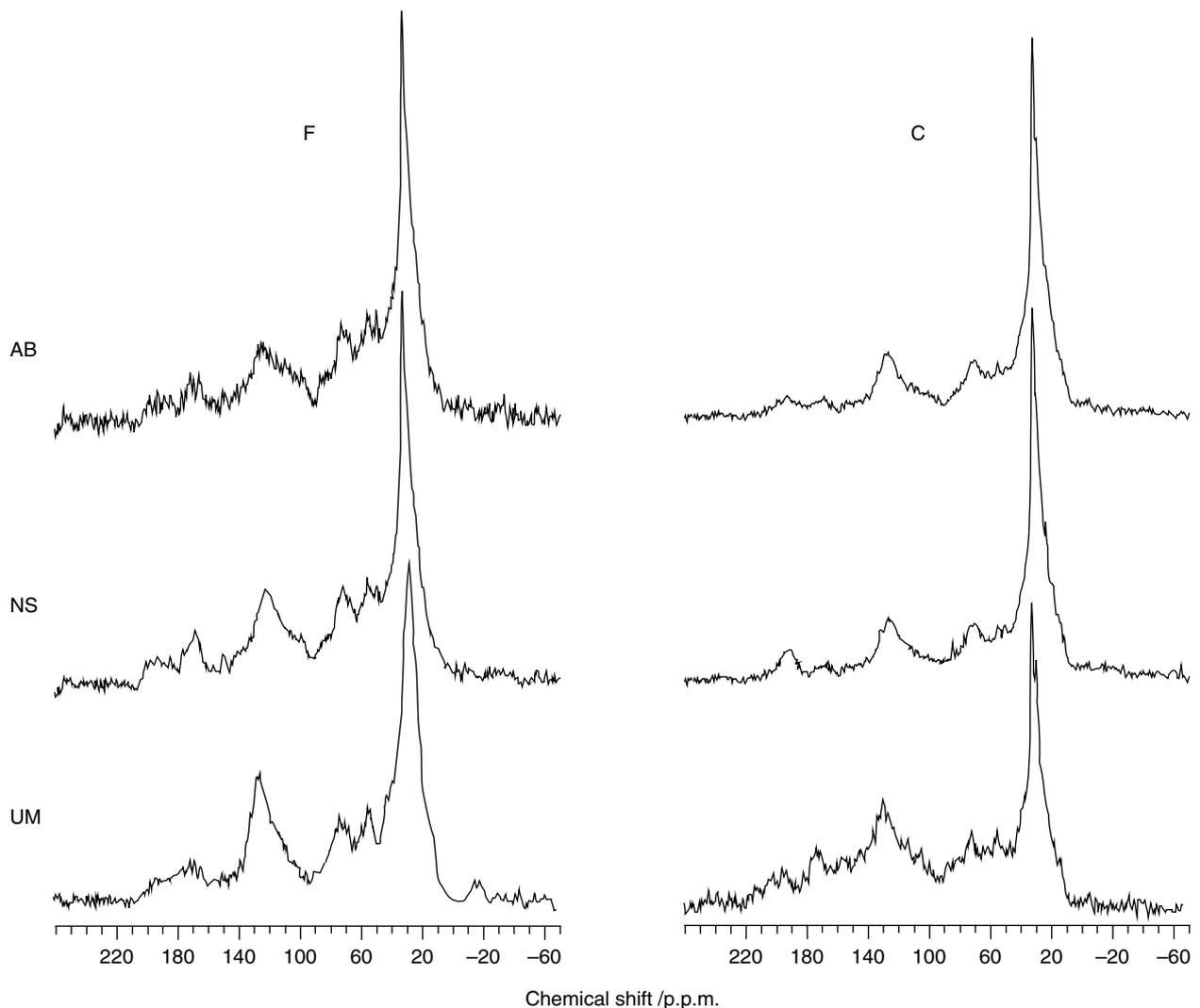


Figure 2 CPMAS ^{13}C -NMR spectra of humic extract (HE) fractions extracted from Nigerian soils (AB, Abakiliki; NS, Nsukka; UM, Umudike) at forested (F) and cultivated (C) sites.

decomposition are affected by the dominant site-specific characteristics such as temperature, humidity, moisture content, pH and nutrient levels. However, while the climatic parameters of the soils of this study are similar (Spaccini *et al.*, 2001), their texture and mineralogical composition are different (Table 1), Abakiliki being the least sandy soil with a predominance of mica in its fine particles, in comparison with the Nsukka and Umudike ultisols which contained mainly kaolinitic clay. This difference may suggest a site-specific influence of soil inorganic components on the transformation of the humified pool of SOM. The presence of high-surface-area clay minerals in Abakiliki, compared with kaolinitic minerals at Nsukka and Umudike, may selectively protect C-substituted, recalcitrant aromatic and alkyl structures by surface adsorption within the humic-clay complexes (Lichtfouse *et al.*, 1998; Spaccini *et al.*, 2000). Conversely, more water-soluble hydrophilic com-

ponents may have undergone rapid biological oxidation during cultivation. Previous studies also indicated a selective preservation of alkyl components in less soluble soil humic fractions (Kögel-Knabner *et al.*, 1992) and a relation of the most hydrophilic FA fraction to the turnover of fresh OM added to soils (Zalba & Quiroga, 1999; Spaccini *et al.*, 2000).

The ^{13}C -NMR spectra of humic substances extracted with acetone-HCl solution (HE) are shown in Figure 2, whereas the C distributions calculated from spectra are given in Table 4. The spectra reveal a large alkyl signature in the 0–50 p.p.m. region (CH_2 and CH_3 groups in polymethylenic chains) for all locations. This is in agreement with NMR spectra of HE fractions isolated from temperate soils (Piccolo *et al.*, 1998; Spaccini *et al.*, 2000) and confirms the large content of apolar organic components (such as waxes and fatty acids) extracted with this humic fraction. As forested soils were turned over to

Table 4 Distribution of ^{13}C (%) in chemical shift intervals of ^{13}C -NMR spectra of humic fractions (HA, FA, HU, HE) from forested (F) and cultivated (C) sites in Nigeria (standard deviations in parentheses)

Fraction	Site	Chemical shift /p.p.m.					HI ^a	HB ^b	HB/HI ^c
		0–50	50–85	85–105	105–160	160–200			
<i>Abakiliki</i>									
HA	F	37.3 (0.9)	23.2 (0.8)	2.2 (0.1)	0.7 (<0.1)	36.6 (1.4)	62.0	38.0	0.61
	C	48.7 (1.8)	16.1 (0.7)	2.5 (0.1)	1.0 (<0.1)	31.7 (1.2)	50.3	49.7	0.99
FA	F	28.6 (0.8)	37.8 (1.1)	5.0 (0.1)	0.1 (<0.1)	28.6 (0.8)	71.4	28.6	0.40
	C	29.1 (1.0)	23.8 (1.1)	7.6 (0.2)	0.1 (<0.1)	39.5 (1.8)	70.9	29.1	0.41
HU	F	31.4 (0.7)	23.0 (1.0)	5.4 (0.1)	5.4 (0.2)	34.8 (1.3)	63.2	36.8	0.58
	C	41.0 (1.2)	15.0 (0.8)	4.0 (0.1)	6.0 (0.2)	34.0 (1.4)	53.0	47.0	0.89
HE	F	56.2 (1.8)	19.0 (0.4)	0.3 (<0.1)	18.0 (0.3)	6.5 (0.2)	25.8	74.2	2.87
	C	52.4 (2.2)	16.0 (0.6)	0.2 (<0.1)	15.7 (0.5)	15.7 (0.4)	31.9	68.1	2.13
<i>Nsukka</i>									
HA	F	48.1 (2.0)	22.1 (0.9)	4.3 (0.1)	0.5 (<0.1)	25.0 (0.8)	51.4	48.6	0.94
	C	46.3 (1.4)	20.2 (0.9)	4.1 (0.2)	0.8 (<0.1)	28.6 (0.5)	52.9	47.1	0.89
FA	F	38.6 (1.3)	25.3 (0.9)	5.6 (0.1)	3.2 (0.1)	27.4 (0.5)	58.2	41.8	0.72
	C	35.0 (1.1)	22.8 (0.7)	5.1 (0.2)	0.8 (<0.1)	36.2 (1.3)	64.2	35.8	0.56
HU	F	52.0 (1.7)	15.2 (0.8)	1.6 (<0.1)	3.2 (0.2)	28.0 (0.7)	44.8	55.2	1.23
	C	35.5 (0.8)	10.7 (0.2)	5.8 (0.1)	9.9 (0.6)	38.0 (2.0)	54.5	45.5	0.83
HE	F	54.6 (2.4)	18.6 (0.5)	0.4 (<0.1)	16.2 (0.4)	9.8 (0.3)	28.8	70.8	2.46
	C	55.3 (1.8)	16.2 (0.6)	0.3 (<0.1)	18.7 (0.6)	12.2 (0.5)	28.7	74.0	2.57
<i>Umudike</i>									
HA	F	41.5 (1.9)	19.5 (0.8)	5.5 (0.3)	8.5 (0.6)	25.0 (1.3)	50.0	50.0	1.00
	C	38.2 (0.9)	15.3 (1.0)	5.6 (0.4)	8.4 (0.9)	33.5 (1.9)	54.4	46.6	0.86
FA	F	30.3 (0.9)	32.0 (1.2)	6.6 (0.2)	0.8 (<0.1)	30.3 (1.6)	68.9	31.1	0.45
	C	35.3 (1.5)	25.3 (0.7)	3.2 (0.1)	0.9 (<0.1)	35.3 (1.1)	63.8	36.2	0.57
HU	F	31.1 (1.1)	24.1 (0.8)	10.4 (0.3)	11.6 (0.5)	22.8 (0.6)	57.3	42.7	0.75
	C	35.3 (1.8)	13.4 (0.2)	2.5 (0.1)	8.4 (0.1)	40.3 (2.2)	56.3	43.7	0.78
HE	F	43.4 (1.0)	25.0 (1.0)	0.2 (<0.1)	16.8 (0.7)	14.7 (0.5)	39.9	60.1	1.51
	C	41.8 (1.2)	23.1 (0.9)	0.3 (<0.1)	20.9 (0.5)	14.0 (0.5)	37.4	62.6	1.68

^aPer cent of hydrophilic carbon: [(50–85) + (85–105) + (160–200)].

^bPer cent of hydrophobic carbon: [(0–50) + (105–160)].

^cDegree of hydrophobicity.

cultivation, the HE fraction at all locations revealed a constant content of the aliphatic-C (0–50 p.p.m.) and a significant decrease of the C-N-C (50–85 p.p.m.) region (Table 4). The aromatic-C (105–160 p.p.m.) was enhanced significantly at Nsukka and Umudike and decreased at Abakiliki. The HE fraction showed a significant increase in oxidation (carboxylic-C, 160–200 p.p.m.) at all sites with the exception of Umudike.

The large content of hydrophobic carbon (aromatic-C + aliphatic-C) of the HE fraction resulted in a degree of hydrophobicity (HB/HI) substantially larger than for the rest of the humic fractions (Table 4). Moreover, the hydrophobicity of the HE fraction increased with cultivation except for Abakiliki. Lichtfouse *et al.* (1998) indicated that alkyl compounds constitute the basic component of the recalcitrant OM strictly associated with the finest (< 2 μm) soil particles. The HE fraction appears very similar to the chemical characteristics of 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). 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 of the HE fraction are also likely to represent the most persistent fraction of stable OM in these tropical soils based on a mechanism of selective preservation of hydrophobic components (Piccolo, 1996; Lichtfouse *et al.*, 1998). The stability of apolar material in soils is attributed to the entropy-driven separation of this hydrophobic fraction from water and, in turn, from microbial activity (Piccolo, 2002).

The significant quantitative decrease of HE, followed in order of magnitude by that of HA, FA and HU (Table 2), when tropical forested soil is increasingly disturbed by farming practices is accompanied by significant chemical changes in the

nature of the residual humic materials. The humic substances of the tropical soils of this study have shown a site-specific tendency to stabilize their hydrophobic constituents with cultivation, as a function of soil mineralogy. This is generally reflected in the intrinsic hydrophobicity (HB/HI) of the humic fractions. However, the concomitant variation of more labile hydrophilic compounds or the increase of oxidized components might have altered the consistent degree of hydrophobicity in some other humic fractions.

Previous work suggested that the hydrophobic components 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 smaller is the mineralization of bio-labile compounds and the release of CO₂ (Piccolo *et al.*, 1999; Spaccini *et al.*, 2000). The loss of hydrophobic components due to disruption of soil macroaggregates and exposure of organic compounds to biotic and abiotic oxidation may be conducive to less stable humic associations and more fragile soil structures (Piccolo, 2002). The results on Nigerian soils appear to confirm these previous findings in that the alteration of the OM status with cultivation caused the loss of its protective properties by generally decreasing the hydrophobicity accumulated under forest and increasing the relative content of oxidation products.

Acknowledgements

This work was supported by the European Union research project STD-TS3*-CT94-0281, 'Evaluation of the stability of soil organic matter (SOM) pools in African agroecosystems for the improvement of a sustainable soil management'. We are grateful to The Abdus Salam International Centre for Theoretical Physics, Trieste, for a Visiting Fellowship (Training and Research in Italian Laboratories) to J.S.C. Mbagwu. The determination of isotopic $\delta^{13}\text{C}$ values at the Université de Bourgogne, Dijon, and CPMAS ^{13}C -NMR spectra at the Università di Roma 'Tor Vergata', Rome, are gratefully appreciated.

References

- Agbim, N.N. 1987. Dry season decomposition of leaf litter from five common plant species of West Africa. *Biological Agriculture and Horticulture*, **4**, 213–224.
- Alegre, J.C. & Cassel, D.K. 1986. Effect of land clearing methods and post clearing management on aggregate stability and organic carbon content of a soil in the humid tropics. *Soil Science*, **142**, 289–295.
- Asadu, C.L.A., Diels, J. & Vanlauwe, B. 1997. A comparison of the contributions of clay, silt and organic matter to the effective CEC of soils of sub-Saharan Africa. *Soil Science*, **162**, 785–794.
- Ayanaba, A. & Jenkinson, D.S. 1990. Decomposition of carbon-14 labeled ryegrass and maize under tropical conditions. *Soil Science Society of America Journal*, **54**, 112–115.
- Bernoux, M., Cerri, C.C., Neill, C. & de Moraes, J.F.L. 1998. The use of stable carbon isotopes for estimating soil organic matter turnover rates. *Geoderma*, **82**, 43–58.
- Boutton, T.W., Archer, S.R., Midwood, A.J., Zitzer, S.F. & Bol, R. 1998. $\delta^{13}\text{C}$ values of soil organic carbon and their use in documenting vegetation change in a subtropical savanna ecosystem. *Geoderma*, **82**, 5–41.
- Conte, P., Piccolo, A., van Lagen, B., Buurman, P. & de Jager, P.A. 1997. Quantitative aspects of solid-state ^{13}C -NMR spectra of humic substances from soils of volcanic systems. *Geoderma*, **80**, 327–338.
- Desjardins, T., Andreux, F., Volkoff, B. & Cerri, C.C. 1994. Organic carbon and ^{13}C contents in soils and soil size-fractions, and their changes due to deforestation and pasture installation in eastern Amazonia. *Geoderma*, **61**, 103–118.
- Dutartre, P., Bartoli, F., Andreux, F., Portal, J.M. & Ange, A. 1993. Influence of content and nature of organic matter on the structure of some sandy soils from West Africa. *Geoderma*, **56**, 459–478.
- Feller, C. & Beare, M.H. 1997. Physical control of soil organic matter dynamics in the tropics. *Geoderma*, **79**, 69–116.
- Flexor, J.-M. & Volkoff, B. 1977. Distribution de l'isotope stable ^{13}C dans la matière organique d'un sol ferrallitique de l'État de Bahia (Brésil). *Comptes Rendus à l'Académie de Sciences, série D, Paris*, **284**, 1655–1657.
- Gee, G.W. & Bauder, J.W. 1986. Particle-size analysis. In: *Methods of Soil Analysis: Part 1, Physical and Mineralogical Methods*, 2nd edn (ed. A. Klute), pp. 383–411. American Society of Agronomy, Madison, WI.
- Gregorich, E.G., Ellert, B.H. & Monreal, C.M. 1995. Turnover of soil organic matter and storage of corn residue carbon estimated from natural C-13 abundance. *Canadian Journal of Soil Science*, **75**, 161–167.
- Kögel-Knabner, I., de Leeuw, J.W. & Hatcher, P.G. 1992. Nature and distribution of alkyl carbon in forest soil profiles: implications for the origin and humification of aliphatic biomacromolecules. *Science of the Total Environment*, **118**, 175–185.
- Lepsch, I.F., Menk, J.R.F. & Oliveira, J.B. 1994. Carbon storage and other properties of soils under agriculture and natural vegetation in Sao Paulo State, Brazil. *Soil Use and Management*, **10**, 34–42.
- Lichtfouse, E., Chenu, C., Baudin, F., Leblond, C., Da Silva, M., Behar, F. *et al.* 1998. A novel pathway of soil organic matter formation by selective preservation of resistant straight-chain biopolymers: chemical and isotope evidence. *Organic Geochemistry*, **28**, 411–415.
- Martin, A., Mariotti, A., Balesdent, J., Lavelle, P. & Vuattoux, R. 1990. Estimate of organic matter turnover rate in a savanna soil by ^{13}C natural abundance measurements. *Soil Biology and Biochemistry*, **22**, 517–523.
- Nadelhoffer, K.J. & Fry, B. 1988. Controls on natural nitrogen-15 and carbon-13 abundances in forest soil organic matter. *Soil Science Society of America Journal*, **52**, 1633–1640.
- Nelson, D.W. & Sommers, L.E. 1982. Total carbon, organic carbon, and organic matter. In: *Methods of Soil Analysis: Part 2, Chemical and Microbiological Properties*, 2nd edn (eds A.L. Page, R.H. Miller & D.R. Keeney), pp. 539–579. American Society of Agronomy, Madison, WI.
- Piccolo, A. 1996. Humus and soil conservation. In: *Humic Substances in Terrestrial Ecosystems* (ed. A. Piccolo), pp. 225–264. Elsevier, Amsterdam.

- Piccolo, A. 2002. The supramolecular structure of humic substances. A novel understanding of humus chemistry and implications in soil science. *Advances in Agronomy*, **75**, 57–134.
- Piccolo, A. & Conte, P. 2003. Comments on *Modern Analytical Studies of Humic Substances* by Hatcher *et al.* *Soil Science*, **168**, 73–74.
- Piccolo, A., Conte, P., Scheunert, I. & Paci, M. 1998. Atrazine interactions with soil humic substances of different molecular structure. *Journal of Environmental Quality*, **27**, 1324–1333.
- Piccolo, A., Spaccini, R., Haberhauer, G. & Gerzabek, M.H. 1999. Increased sequestration of organic carbon in soil by hydrophobic protection. *Naturwissenschaften*, **86**, 496–499.
- Preston, C.M. & Newman, R.H. 1995. A long term effect of N fertilization on the ^{13}C -CPMAS-NMR of de-ashed soil humin in a second-growth Douglas-fir stand of coastal British Columbia. *Geoderma*, **68**, 229–241.
- Puget, P., Chenu, C. & Balesdent, J. 1995. Total and young organic matter distributions in aggregates of silty cultivated soils. *European Journal of Soil Science*, **46**, 449–459.
- Spaccini, R., Piccolo, A., Haberhauer, G. & Gerzabek, H. 2000. Transformation of organic matter from maize residues into labile and humic fractions of three European soils as revealed by ^{13}C distribution and CPMAS-NMR spectra. *European Journal of Soil Science*, **51**, 583–594.
- Spaccini, R., Zena, A., Igwe, C.A., Mbagwu, J.S.C. & Piccolo, A. 2001. Carbohydrates in water-stable aggregates and particle size fractions of forested and cultivated soils in two contrasting tropical ecosystems. *Biogeochemistry*, **53**, 1–22.
- Spaccini, R., Mbagwu, J.S.C., Igwe, C.A., Conte, P. & Piccolo, A. 2004. Carbohydrates and aggregation in lowland soils of Nigeria as influenced by organic inputs. *Soil and Tillage Research*, **75**, 161–172.
- Thomas, G.W. 1982. Exchangeable cations. In: *Methods of Soil Analysis: Part 2, Chemical and Microbiological Properties*, 2nd edn (eds A.L. Page, R.H. Miller & D.R. Keeney), pp. 159–165. American Society of Agronomy, Madison, WI.
- Wedin, D.A., Tieszen, L.L., Dewey, B. & Pastor, J. 1995. Carbon isotope dynamics during grass decomposition and soil organic matter formation. *Ecology*, **76**, 1383–1392.
- Zalba, P. & Quiroga, A.R. 1999. Fulvic acid carbon as a diagnostic feature for agricultural soil evaluation. *Soil Science*, **164**, 57–61.
- Zech, W. & Guggenberger, G. 1996. Organic matter dynamics in forest soils of temperate and tropical ecosystems. In: *Humic Substances in Terrestrial Ecosystems* (ed. A. Piccolo), pp. 101–170. Elsevier, Amsterdam.
- Zech, W., Senesi, N., Guggenberger, G., Kaiser, K., Lehmann, J., Miano, T.M. *et al.* 1997. Factors controlling humification and mineralization of soil organic matter in the tropics. *Geoderma*, **79**, 117–161.