Biogeochemical Assessment of Resilient Humus Formations from Virgin and Cultivated Northern Botswana Soils

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Humic matter in virgin and cultivated Vertisols and Arenosols from Pandamatenga (northern Botswana) has been studied by chemical fractionation and visible and infrared derivative spectroscopies. The in vitro soil respiration was also determined. Soils contained <15 g of total C kg^{-1} and displayed scant mineralization activity. In Vertisols, cultivation has led to scarce significant changes in humus characteristics, pointing to a noteworthy resilience of the organic matter. Humic acids showed a very dark color, indicating a large concentration of aromatic structures and stable free radicals. Infrared spectra were featureless and alike. This strong structural stability is also suggested by Curie-point pyrolysis of humic acids, which failed to yield substantial amounts of diagnostic products, mainly in the case of Vertisols. Some unexpected similarities between Vertisols and Arenosols indicate that the influence of external factors on the humic acid formation processes prevails on that of the geological substrate. In Pandamatenga soils only small amounts of recalcitrant C and N forms are sequestered; they represent stable pools relatively independent from short- or medium-term climatic changes or management practices.

KEYWORDS: Arenosol; C sequestration; humic acid; Kalahari; Okavango delta; organic matter; Vertisol

INTRODUCTION

There is little information available on the mechanisms controlling soil organic matter accumulation in savanna soils, although such formations cover extensive tropical and subtropical zones (*1*, *2*). This knowledge is required for both the assessment of agricultural sustainability levels and the ecological modeling of tropical ecosystems. In northern Botswana, a considerable area of woodland and brushwood has been cleared and cultivated.

Apart from clayey soils (Vertisols), extensive soil formations in northern Botswana derive from Kalahari sands (Arenosols) with a low agronomic potential. In these areas human pressure is still relatively low. However, the establishment of stable, dense plant communities is often limited by exploitation of firewood, grazing, or the destructive effects on the tree layer by large vertebrates and periodic burning. Even though the above constraints do not favor the accumulation of soil organic matter, there is some local influence of waterlogging in the wet seasons, which is presumed to play a relevant role in the selective preservation of organic matter in tropical conditions.

The present study deals with an assessment of humification processes based on comparisons between representative eco-systems under sandy and clay soils in Pandamatenga (northern Botswana). The objectives are (a) to provide original, preliminary data on the characteristics of the major forms of organic matter accumulated in these soils and on (b) the effect of clearing and cultivation on Pandamatenga Vertisols and (c) to get some insight on the balance between biotic and abiotic factors in the biogeochemical performance and carbon sequestration in the two different types of ecosystems considered.

MATERIALS AND METHODS

Topsoils of different Arenosols and Vertisols located in contrasting, representative ecosystems from northern Botswana were collected as part of an EU program on sustainable management of southern African savannas. Sampling was conducted on sandy (three sites) and clayey soil areas (two sites). Soil litter was removed, and the samples (three spatial replications corresponding to combined samples obtained by mixing in the field three subsamples ∼20 m apart) were collected from the upper 10 cm. No attempt was made to collect any soil material from the underlying horizons because the observation of soil profiles suggested no well-defined horizon pattern, gradual transition in the humus concentration, and a comparatively lower C content in the 20–30 cm layer, unsuitable for preparative isolation of representative amounts of humic substances. To prevent sample transformation during storage, the soil material collected with a spade in the field was transported in polyethylene bags and desiccated by sun and air exposure during the field trip stops; complete desiccation took 4 days in the case of samples from waterlogged sites, whereas samples from sandy savanna soils and Vertisols were practically dry when collected.

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The least significant difference (LSD) test (*3*) has been used to compare the statistical significance of the differences between the spatially replicated samples.

Study Areas. The main study area was Pandamatenga, with control research areas in eastern Ngamiland in northern Botswana, including the Okavango delta. The vegetation is primarily of the semiarid savanna woodland type and is mainly influenced by soil type, drainage, herbivores, and fires (*4*). Pandamatenga is the area most suited to dryland crop production in Botswana, with an average rainfall of at least 550 mm. Commercial farming is extensively practiced in the central and southern clayey plains. The crops grown include maize, cotton, and sunflower. The farms are adjacent to forest reserves, wildlife management areas, and other areas of wildlife importance on the Zimbabwean side of the border.

The vegetation in the southern part of Ngamiland is mainly shrub and tree savanna dominated by species such as *Terminalia sericea* Burch. ex DC., *Lonchocarpus nelsii* (Schinz) Heering and Grimme subsp. *nelsii*, and *Acacia erubescens* Welw. ex Oliver. In the eastern part of the delta, vegetation is shrub and tree savanna, and the common tree species are *Colophospermum mopane* Kirk ex Benth, *T. sericea*, and *L. nelsii*. The sites studied included sandy soils (Arenosols collected along an edaphic moisture gradient) and clay soils (Vertisol samples collected from both virgin and cultured fields).

Arenosols. *Site SS (Sandy Savanna).* The soils at this site (Xhana Settlement, 40 km east of Maun, along Maun−Francistown road) correspond to Dystric Arenosols developed from Kalahari sands with a dense herbaceous (Poaceae) layer, high bush, and sparse trees dominated by *Acacia* sp. The rain averages 463 mm from October/November to March/April, and the temperature ranges annually from 19 to 33 °C (*5*). This soil presents a presumably high mineralization rate, because the open vegetation type and the low abundance of colloidal minerals would not favor the stabilization of the organic matter.

Site OS (Okavango Station). Soil samples were taken from an Arenosol near the Harry Oppenheimer Okavango Research Center, located at Nxaraga on Chief's Island. Here humid spots lead to the formation of blackish (when wet) soil material. Because the soil mineral fraction consisted practically of quartz sand, no typical soil hydromorphic features (e.g., grayish or reddish layers or spots in the soil profile) were recognized. The site receives 600 mm of rain in a discontinuous period of ∼6 months. Much of the water comes from flooding by the Okavango channels starting in July/August, causing waterlogging of the sampling site. Vegetation consisted of a mixture of palustric and xerophytic grassland in addition to some bush and low-density Fabaceae forest, which to a large extent has been destroyed by elephant grazing.

Site OC (Okavango Channels). Soils in this area are developed on sandy river sediments with dense vegetation of Cyperaceae, Juncaceae, and typical hydrophytic species (mainly *Nymphaea*). This site is comparable to OS, but waterlogging is practically constant for the whole wet season (∼90 days per year).

Vertisols. *Site VV (Virgin Vertisol).* Black Vertisol was obtained close to the Masedi cotton farm. It is developed in the typical savanna forest environment including dense brushwood in addition to a dense herbaceous layer.

Site CV (Cleared Vertisol). This was obtained from a cultivated field that had characteristics similar to those of site VV. The cultivated field is a commercial farm (cereal/cotton), separated by ∼100 m from VV forest by a road. This soil had been cleared ∼50 years ago. For the past 10 years it has been managed with reduced tillage practices including NPK fertilization and controlled burnings.

General Analyses. The air-dried soil samples were crushed on a table with a wooden cylinder; root and rock fragments were removed, and the soil material was sieved to 2 mm. The pH was measured in a 1:2.5 soil-to-water suspension. The total N was determined by micro-Kjeldahl digestion and the available P according to the Bray and Kurtz (*6*) method. Available K, Ca, and Mg were extracted with 1 M NH₄-OAc (pH 7) and the available micronutrients with diethylenetriamine-pentaacetic acid (*7*). Effective cation exchange capacity (ECEC) was obtained by adding the exchangeable acidity to the exchangeable cations determined with 1 M NH₄OAc (*8*). Clay minerals were identified by X-ray diffraction and infrared absorption.

In the case of whole soils and soil extraction residues, C was measured in samples ground to a fine powder with an agate mortar by dry combustion using a Wösthoff furnace attached to a Carmhograph-12 gas analyzer, whereas the extractive organic fractions were quantified in C by wet chemical oxidation according to the Walkley and Black method (*9*).

Humus Fractions. Isolation and quantitative analysis of the humus fractions were carried out by using the different procedures described by Dabin, Merlet and Duchaufour, and Jacquin (*10−12*). The fractionation method used involves previous isolation of soil lipid (petroleum ether 60−70 °C, 12 h in Soxhlet extractor, further dehydration of the extract with anhydrous Na₂SO₄, and removal of the solvent at reduced pressure), followed by physical separation of the particulate soil fraction consisting of the not-yet decomposed organic remains (free organic matter; flotation in 0.1 M H₃PO₄, followed by centrifugation and repeated washing with water before the particulate fraction was dried). Then, the soil residue (I) was successively extracted with 0.1 M Na₄P₂O₇ and 0.1 M NaOH, five times each. The dark brown supernatant solution (total humic extract) was isolated by centrifugation and combined with the yellowish extract from the H₃PO₄ separation. The remaining soil residue (II) was subjected three times to a treatment with a mixture of 60 mM Na₂S₂O₄ and 1 M HCl−HF (1:1) at 60 °C to remove the aluminum and iron oxides and the clay, respectively, and then extracted with 0.1 M NaOH to obtain the humic colloids, which were in stable association with the mineral matrix (insolubilized extractable humin). Aliquots of the humic extract previously separated were precipitated with H₂SO₄ (50% by volume), centrifuged, and used for quantitative determination of the acid-soluble (fulvic acid) and the acid-insoluble (humic acid) fractions. Aliquots of the extracts containing insolubilized extractable humin were processed in the same way.

After the extraction, a fraction referred to as light humin (70−100 g of C kg⁻¹) was separated by siphoning and sieving the final residue (III) of some Arenosols, corresponding to the blackish finely particulate layer at the top of the extraction residue previously suspended in water.

Light and scanning electron microscope observations, the latter performed in a Zeiss DSM 960, using graphite-coated soil samples and an electron dispersive spectroscopy system (EDS) for element mapping in the case of amorphous particles, showed their homogeneous structure, suggesting a stable organomineral fraction with no possibility of further fractionation based on physical procedures.

For further structural characterization of the humic acids, they were purified as follows: the remaining humic extract was precipitated with HCl, de-ashed with 1 M HCl−HF treatments at room temperature, redissolved in 0.5 M NaOH, centrifuged at 43500*g*, reprecipitated, dialyzed in cellophane bags until total elimination of the salts (AgNO₃ test) introduced during the extraction procedure, and finally freeze-dried.

Soil Respiratory Activity. To obtain comparative data about the intrinsic biodegradability of the soil organic matter, the in vitro respiratory activity was measured as the CO₂ released by samples of 20 g of soil homogenized to 2 mm and moistened to 60% of the soil water-holding capacity at atmospheric pressure and at 24 ± 1 °C. The CO₂ produced by microbial metabolism was measured periodically during 70 days with a Carmhograph-12 (Wösthoff) gas analyzer. The samples were kept in 250 mL Erlenmeyer flasks with rubber stoppers provided with polyethylene inlet and outlet tubes.

During the incubation period, the flasks were kept in an oven and the atmosphere in them was analyzed daily by connecting the outlet tube to the CO₂ analyzer and the inlet tube to a soda-lime column (*13*). The method used and the ratios calculated were similar to those described by Chone et al. (*14*). The mineralization of the organic matter was expressed both in absolute terms (milligrams of carbon released per soil weight unit per day) and in relative terms, that is, taking into account that each soil had a different organic matter content (milligrams of carbon released per kilogram of soil carbon per day). The former will be referred to as mineralization rates and the latter as mineralization coefficients.

Humic Acid Characteristics. The determination of carbon, nitrogen, and hydrogen was carried out with a Carlo Erba CHNS-O-EA1108 microanalyzer, using ∼7 mg of humic acid. The percentages were calculated on an ash-free basis. The oxygen was calculated by

Table 1. General Analytical Characteristics of the Topsoil (0–10 cm) in the Pandamatenga Savanna

	Arenosols			Vertisols		
	SS	OS	OC	VV	CV	LSD[a]
very coarse sand (2–1 mm) (g kg^{-1})	2	3	2	9	12	1
coarse sand (1–0.5 mm) (g kg^{-1})	17	29	25	26	27	8
medium sand (0.5–0.2 mm) (g kg^{-1})	511	365	390	73	71	41
fine sand (0.2–0.05 mm) (g kg^{-1})	419	402	333	60	54	27
very fine sand (0.05–0.02 mm) (g kg^{-1})	19	75	67	38	36	14
total sand (2–0.02 mm) (g kg^{-1})	968	874	817	206	202	89
silt (0.02–0.002 mm) (g kg^{-1})	3	35	45	134	122	17
clay (<0.002 mm) (g kg^{-1})	15	15	81	627	660	82
pH	8.1	7.7	8.6	8.3	8.2	0.2
ECEC[b] (cmol$_c$ kg^{-1})	3.8	5.0	8.1	30.1	76.5	22
base saturation (%)	98	89	97	99	81	19
electrical conductivity (μS cm^{-1})	204	151	256	200	190	59
soil C (g kg^{-1})	5.8	12.0	13.5	10.1	8.3	1.1
soil N (g kg^{-1})	0.4	0.8	1.0	0.5	0.6	0.3
C/N ratio	15.7	14.8	13.1	20.0	14.5	7.1
P (mg kg^{-1})	17.5	4.5	36.4	4.3	2.8	14
K (mg kg^{-1})	15	71	172	246	403	25
Ca (mg kg^{-1})	470	820	2180	7610	7135	1352
Mg (mg kg^{-1})	50	105	137	1262	1462	190
Fe (mg kg^{-1})	8.5	12.6	15.2	63.3	107.0	58
Mn (mg kg^{-1})	6	42	86	413	326	219
Zn (mg kg^{-1})	0.5	0.4	0.8	0.4	0.4	0.3
Cu (mg kg^{-1})	0.4	0.9	0.9	5.6	5.0	1.3

[a] LSD = least significant difference between replicated samples taken at the five sites studied. [b] ECEC = effective cation exchange capacity.

difference. The ash content was determined after combustion in an electric furnace at 550 °C for 8 h.

To determine the optical density of the humic acids, visible spectra were acquired from solutions of 100 mg of carbon L^{-1} in 0.02 M NaHCO$_3$ [i.e., about half of the concentration suggested by Kononova (*15*) on the basis of the color intensity of "average" humic acids]. The IR spectra were acquired with a Bruker IFS28 Fourier transform spectrophotometer using KBr pellets with 2 mg of humic acid and oven-dried. As usual in humic acids with a deep diagenetic transformation, the IR spectra consisted of weak, overlapped absorption bands, and they were subjected to a digital procedure for resolution enhancement based on the subtraction of the raw spectrum from a positive multiple of its second derivative (*16*, *17*). The peak intensities in the original spectra were also tabulated after their absorption values were divided by that corresponding to the band at 1620 cm^{-1}. This band was selected because of its high intensity and unambiguous detection in all spectra, being a multiple-origin band for several typical humic acid structures, mainly those present in the aromatic network (*18*).

Analytical pyrolysis was carried out with a Curie-point pyrolyzer unit (Horizon Instruments) attached to a Varian Saturn 2000 gas chromatography–mass spectrometry (GC-MS) system. Samples on ferromagnetic wires were heated at Curie temperature of 510 °C for 5 s. The interface temperature of the pyrolysis unit was set at 250 °C, and the GC oven was programmed from 50 to 100 °C at 32 °C min^{-1}

and then up to 320 °C to a rate of 6 °C min^{-1}. The injector, equipped with a liquid CO$_2$ cryogenic unit, was programmed from −30 °C (1 min) to 300 °C at 20 °C min^{-1}. For chromatographic separation a 25 m × 0.32 mm fused-silica capillary column coated with CPSil (film thickness = 0.4 mm) and He as carrier gas was used. Compound identification was based on literature data and further comparisons with mass spectral libraries (*19*).

RESULTS AND DISCUSSION

General Characteristics. The main physicochemical characteristics of the soils are given in **Table 1**. Vertisols at Pandamatenga are slightly to moderately alkaline. Their effective cation exchange capacity shows values >30 cmol$_c$ kg^{-1}, a common feature in these montmorillonitic soils. The exchange complex is dominated by calcium, and base saturation approaches 100%. From the electrical conductivity results, the soils can be classified as nonsaline. Total nitrogen content in the topsoils is <1 g kg^{-1}. Except in SS and OC sites, available P is deficient, whereas the amounts of K, Ca, and Mg can be considered adequate for plant growth. The Vertisols contained up to 413 mg of Mn kg^{-1}, which is probably in part responsible for their gray color. In particular, dark color in soils having medium-to-low organic matter content is frequently a subject of controversy in the scientific literature. Thus, although some authors have demonstrated that the soil color is weakly related with the total organic matter, a higher correlation was obtained when organic pigments contributing to soil color were considered, which is the case with the humic acid (*20*) as it has occurred in our samples (**Table 2**). It has also been reported (*21*) that a general darkening may be due to either cementation or aggregation of very small crystals into hard dense masses. In addition, the structural disordering could also contribute to the dark color. From this viewpoint, very high concentrations of iron oxides in Vertisols would be responsible for their blackish color. These results contrast with those reported by Schmidt et al. (*22*), who, after excluding alternative explanations based on soil mineralogy, found in a color sequence from dark gray to black soils in some German chernozemic soils (on average 17 g of C kg^{-1}) that the intensity of the color depended on the concentration of "charred organic carbon".

The Arenosols have slightly to moderately alkaline soil pH values. The ECEC is very low (<8 cmol$_c$ kg^{-1}) as it is the amount of total N, available P and K, secondary nutrients (Ca and Mg), and available micronutrients.

As expected, the lowest carbon values corresponded to the freely drained sandy soil, whereas some significantly ($P < 0.05$) higher carbon concentration values were observed with increasing waterlogging. The C/N ratios were similar in all of the soils.

No general agreement exists on the role of clay content in the accumulation of soil organic carbon. Nichols (*23*) found strong positive correlation between clay content and soil C

Table 2. Organic Fractions in Savanna Soils (0–10 cm) from Northern Botswana

				g of C kg^{-1} soil					
soil	lipid	free organic matter	total extractable humus	humic acid	fulvic acid	humic acid/ fulvic acid ratio	insolubilized extractable humin	light humin	nonextractable humin
SS	0.46	1.80	3.4	2.1	1.2	1.75	<0.1	<0.1	0.2
OS	0.36	1.26	4.3	3.1	1.3	2.38	1.6	3.0	1.5
OC	0.39	0.82	5.9	4.2	1.7	2.47	3.2	3.2	<0.1
VV	0.06	0.18	6.7	5.3	1.4	3.79	<0.1	<0.1	3.1
CV	0.05	0.03	5.3	4.2	1.1	3.82	<0.1	<0.1	2.9
LSD[a]	0.07	0.53	2.8	1.9	0.8	2.38	<0.1	<0.1	1.9

[a] LSD = least significant difference between replicated samples taken at the five sites studied.
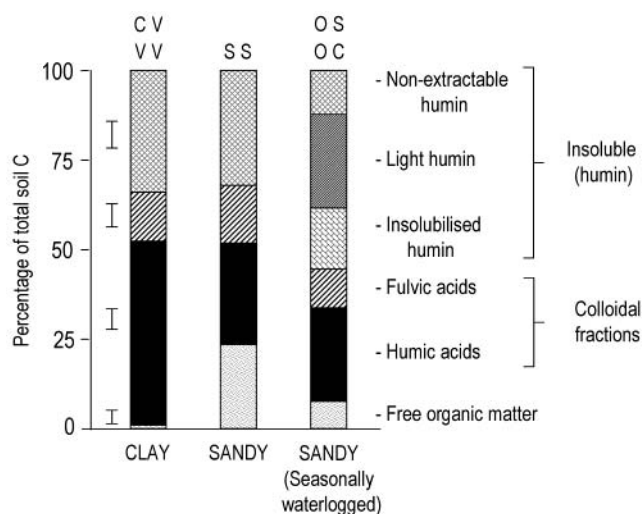
Figure 1. Average values for the major humus fractions in sandy and clayey soils from northern Botswana. Error bars indicate the least significant difference between average values determined from replicated sampling.

concentration, whereas Sims and Nielsen (*24*) found the opposite. Davidson (*25*) indicates that in addition to soil clay other factors such as soil wetness play an important role in determining stocks of soil organic C. The analyses of the soil organic matter fractions (**Table 2**) show an extremely low proportion of fresh organic matter in Vertisols, lower than in Arenosols, pointing to a possible effect of clay on the preferential accumulation of soil carbon as humic acids (**Figure 1**). At this point it should be taken into account that in tropical soils fire may control the biodegradability of the organic matter. In such soils, black C particles can be strongly retained by amorphous colloidal minerals, leading to a recalcitrant soil C fraction that remains in the profile for decade or century time scales (*26*).

In both soil types, the proportion of plant residues (free organic matter) amounts to < 25%; most of the carbon was present as humic substances (humic acid and fulvic acid). A low relative amount of fulvic acid (fraction of low molecular weight) was also observed, thus showing the preferential accumulation of the more complex fraction (humic acid).

As opposed to most soils developed under temperate climates, no significant amount of humic substances was obtained after demineralizing treatments (insolubilized extractable humin). This could point to a strong association of organic and mineral matter (e.g., the insolubilized humic substances cannot be removed by the drastic $Na_2S_2O_4$ and HF−HCl treatments, but they remain in the final extraction residue amounting to up to one-third of total soil C), which did not seem to occur in the Arenosols. The hydromorphic Arenosols also contain a particulate, dark organomineral fraction, referred to above as light humin. This soil fraction is completely amorphous under both light and scanning electron microscopes. Also, the examination of the particles by EDS indicated an isotropic distribution of the major elements including carbon. The IR spectrum (not shown) displays an overlapping of the typical bands of smectites and humic substances. It is likely that a portion of the clay−organic matter complexes in the waterlogged soils under study could not be removed with standard procedures (treatments with HF−HCl solutions), so that in the Arenosols, most humin could accumulate as the above recalcitrant (subfossil) silty amorphous particulate fraction. The Arenosols contained a comparatively high amount of non-decomposed plant residues. In fact, in these soils the higher the influence of waterlogging (SS < OS < OC),

the greater the diversity or complexity in the distribution of the total C among the different humus fractions (**Figure 1**). We assume that this may be due to the accumulative effects of seasonal variations of the water regime on the humic substances formation processes.

Because the least significant difference (LSD) test showed that the quantitative differences between the humus fractions within the Vertisols (CV and VV) and those within the Arenosols (OS and OC) affected by the most intense waterlogging were not significant (P < 0.05), these values have been averaged in **Figure 1** to illustrate the three different situations studied. The Arenosols had in appearance the least transformed organic matter of the soils studied: >25% free organic matter and a comparatively low humic acid/fulvic acid ratio. With increasing soil moisture regime (SS < OS < OC), the organic matter composition in these soils changes toward a progressive accumulation of nonextractable organic matter. The OC and OS soils contained the greatest amount of insolubilized ($Na_2S_2O_4$ and HF−HCl) extractable humin, which agrees with the general fact that alternating drastic wetting and desiccation cycles are required for this originally colloidal fraction to be fixed on the clay (*27*). Due to the negligible amount of silt and clay, practically all insoluble organic matter consists of light humin; that is, it remains as black dust on the sand residue.

We found some significant differences in the humus fractions in terms of the soil mineralogical composition. The Vertisols had no free organic matter (a small proportion in VV, where it amounts ∼0.1%). They displayed a large dominance of humic acids versus fulvic acids, which contrasts with those situations where the generation of large amounts of fulvic acids is interpreted as an effect of intense biological activity in tropical soils (*28*). No significant change was observed in the concentration of any humic fraction between the virgin and the cultivated Vertisols. These had between 53 and 76% of the total carbon as extractable organic matter (humic acid plus fulvic acid), the humic acid/fulvic acid ratio ranging from 3.81 to 3.96 (**Table 2**). The proportion of free organic matter was negligible (0.3− 1.8% of the total carbon, **Figure 1**) and so was unexpectedly the proportion of extractable humin.

Additional, significant (P < 0.05) differences between Arenosols and Vertisols were found in the lipid concentration (**Table 2**). In fact, in the Vertisols, we found no substantial accumulation of free lipids, the vegetation type (dominant herbaceous layer and only low-density bush and forest) appearing not to be a major source of this difference. This low relative concentration of lipids in the Vertisols agrees with the potential of smectitic clay for its effective fixation, therefore becoming nonextractable compounds (*29*).

Soil Respiratory Activity. The mineralization coefficients of the organic matter showed a very small respiratory activity compared to values commonly reported in temperate areas (*30*). Only ∼2 mg of C kg^{-1} of soil C day^{-1} is released as CO_2 under optimum incubation conditions (**Figure 2**). When the soil respiration values are expressed in terms of the soil weight, the data revealed a much higher production of CO_2 in sandy than in clayey soils, which coincided with the slightly higher accumulation of non-decomposed plant debris in the former case. There was some significant increase in the cleared Vertisols with respect to the virgin Vertisols, indicating that the organic matter accumulated in the former is more readily available to microorganisms, as it could correspond to a higher amount of biomass produced in the cultivated soil leading to a substantial incorporation of crop wastes, mainly subterranean parts of field crops.
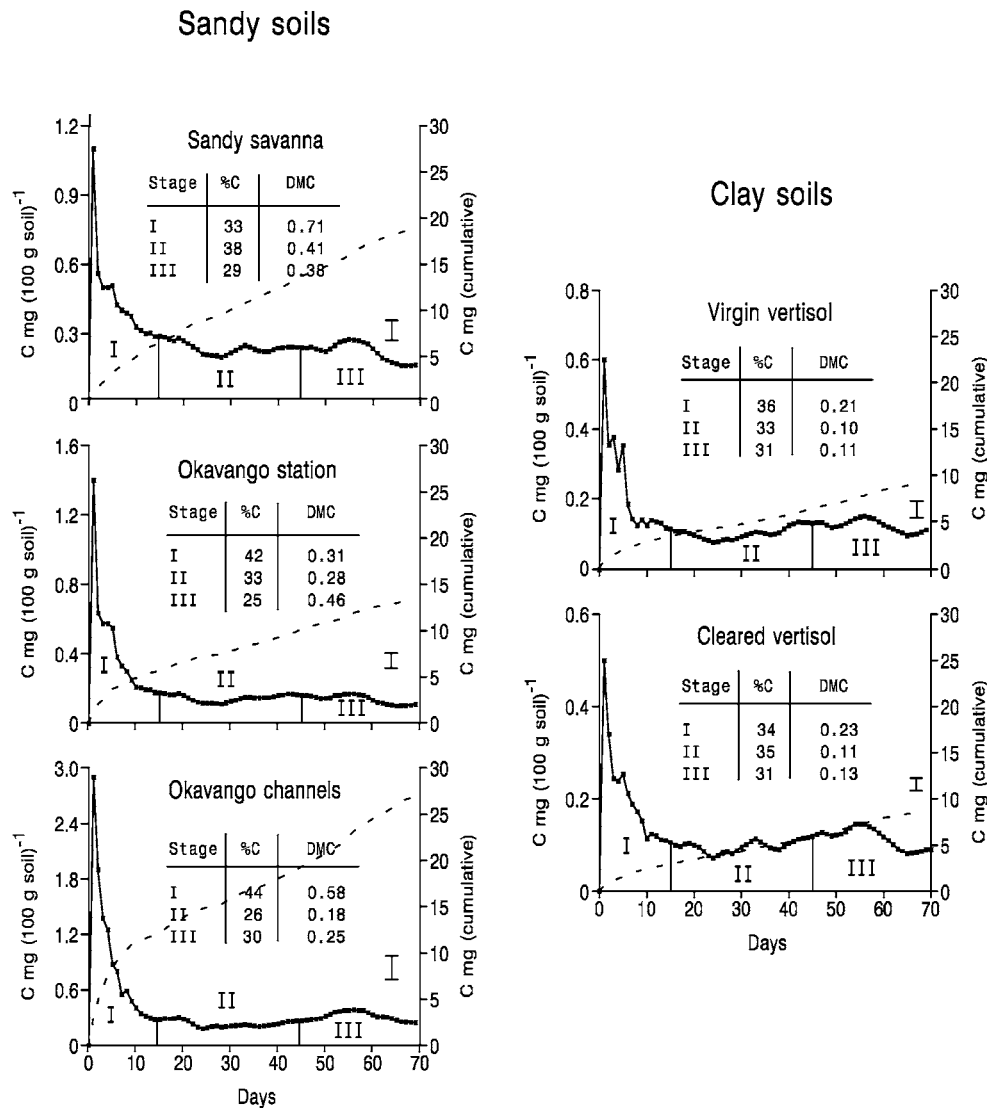
Figure 2. Carbon mineralization curves of soil samples from northern Botswana (average values: the error bars on the curves for daily mineralization show the average variability range among three soil samples collected at different sites). DMC = daily mineralization coefficient [average g of carbon (kg of soil carbon)$^{-1}$ day^{-1}]. Units for the cumulative dotted line are shown in the *y*-axis at the right side, and labels I, II, and III refer to the percentage of the total CO_2 released during the progressive mineralization stages indicated in the plot.

On the other hand, the mineralization coefficients (i.e., referred to the total soil C) were very low in all savanna soils studied, which points to a strong resistance of their organic C to be transformed by microorganisms. The stability of organic matter in the Vertisols suggests that cultivation of these areas has no large effect on the balance of its C with that of the atmosphere.

As a whole, the respiration values are similar to those of most types of fossil or subfossil organic matter (peats, lignites, etc.) (*31*). The probable influence of the contrasted tropical climatic conditions or the exposure of the soil to high temperatures (*32, 33*) could justify the similar low biodegradabilities of C forms in soils developed under contrasting geological substrates, namely, smectites and Kalahari sands. In the latter case intense insolubilization of humic colloids cannot be reasonably invoked to explain such stability. At this point, it should be taken into account that in tropical soils fire may control the biodegradability of the organic matter (*34*). Fine pyrogenic carbonaceous particles with large concentrations of polycyclic compounds in aerosols are generated during savanna fires (*35*) and have been postulated to exert a significant impact in the regional troposphere (*36*) and to be associated to the stability of the humus

formations. In this sense, the frequent anomalous data observed in soils from the Botswana Kalahari transect, where litter and vegetation cover densities were unrelated to soil C concentration (*4*), were attributed to either human-derived land use pressures or natural factors including fire and ungulate grazing and browsing. In other typical savanna formations such as the Brazilian Cerrado (*37*), the presence of recalcitrant organic matter in particulate soil fractions was assigned to charcoal particles after optical microscope examination. In our case, both light and scanning electron microscopes failed to reveal unambiguous charcoal characteristics in the particulate soil fractions including the blackish light humin. These fractions had considerable mineral content and amorphous structure. Such morphology was the same recently described by Poirier et al. (*38*) for the dark angular particles of refractory material isolated from a Ferralic Arenosol under savanna vegetation in Congo. Because the usual demineralizing agents did not reveal any clay-associated extractive humic fraction, the nature of this soil fraction could be related to a pool of still biodegradation-resistant organic particles affected by fires in the past but further subjected to high-performance microbial alteration characteristic of tropical environments (*38*). In this sense, the light humin

Table 3. Elementary Composition (Ash-Free Basis) of Humic Acids from Northern Botswana Savanna Soils

soil	g kg⁻¹				atomic ratios		
	C	H	O	N	C/N	H/C	O/C
SS	557	67	326	47	12	1.46	0.44
OS	524	37	403	35	15	0.86	0.58
OC	541	39	384	35	15	0.88	0.53
VV	560	32	386	20	28	0.70	0.52
CV	548	32	401	18	31	0.72	0.55
LSD[a]	17	7	15	1	1	0.14	0.01

[a] LSD = least significant difference between replicated samples taken at the five sites studied.
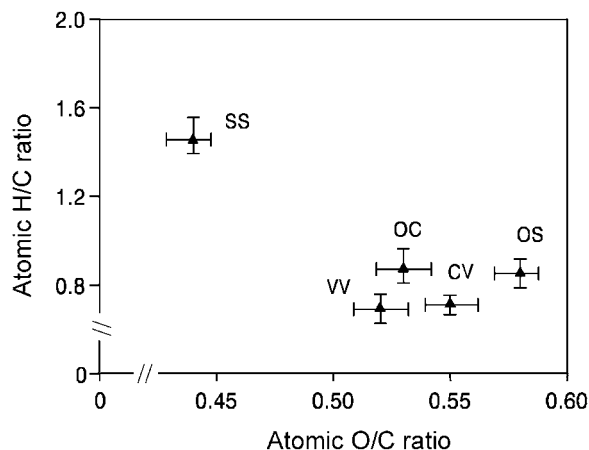

Figure 3. Van Krevelen diagram (atomic H/C vs atomic O/C) of humic acids from northern Botswana. Error bars show the variability between samples taken from different sites from each ecosystem. For soil labels, refer to Materials and Methods.

particles could also be ascribed to the black carbon-containing soil fraction reported in Brazilian Amazon soils (*39*), where similar homogeneous microstructure is explained through additional stabilization by organomineral complexation leading to black carbon embedded within plaques of iron and aluminum oxides on mineral surfaces.

Elementary Composition and Spectroscopic Characteristics of the Humic Acid Fraction. The low C/N ratio of humic acids from the Arenosols compared to the Vertisols (**Table 3**) could correspond to a dominance of a condensed carbon backbone in the latter. In the case of Arenosols there were no significant differences in terms of increasing soil moisture regime (SS < OS < OC), although low C/N ratios are often described in humic acids from reducing environments (*12*). The plot representing the H/C versus O/C atomic ratios of the humic acids (**Figure 3**) (van Krevelen's diagram, *40*) suggests the highly aliphatic character and low concentration of O-containing groups in the SS site, as expected from a situation where very weak organomineral interactions hamper the maturation of the humic matter. In addition, there were small significant differences between the clayey soils and the waterlogged Arenosols (OC and OS). With regard to the effect of cultivation, the only significant ($P < 0.05$) change in the Vertisols consisted of a slight increase in the atomic O/C ratio, interpreted as enhanced biological oxidative processes that might be expected from the mechanical surface mixing produced by the tillage practices.

Most of the spectroscopic characteristics of Pandamatenga humic acids were considerably different from those of temperate soils. This fact is particularly relevant with regard to the optical density of humic acid solutions adjusted to a constant concentra-

Table 4. Spectroscopic Data of Humic Acids from Northern Botswana Savanna Soils

sample	optical density values in the visible range (wavelength, nm)		relative optical densities[a] of the main bands (wavelength, cm⁻¹) in the infrared spectra					
	E_{465} (E_4) (100 mg of C L⁻¹)	E_{465}/E_{665} (E_4/E_6)	3400	2920	1720	1460	1230	1030
SS	0.59	4.45	0.96	0.87	0.86	0.58	0.59	0.51
OS	1.51	3.06	0.94	0.66	0.81	0.49	0.61	0.32
OC	1.70	2.70	0.85	0.71	0.67	0.81	0.66	0.71
VV	1.91	2.84	0.66	0.58	0.80	0.50	0.65	0.25
CV	2.08	2.52	0.62	0.56	0.68	0.50	0.61	0.28
LSD[b]	0.38	0.80	0.04	0.09	0.04	0.03	0.05	0.03

[a] Relative to the intensity of the 1620 cm⁻¹ band. [b] LSD = least significant difference between replicated samples taken at the five sites studied.
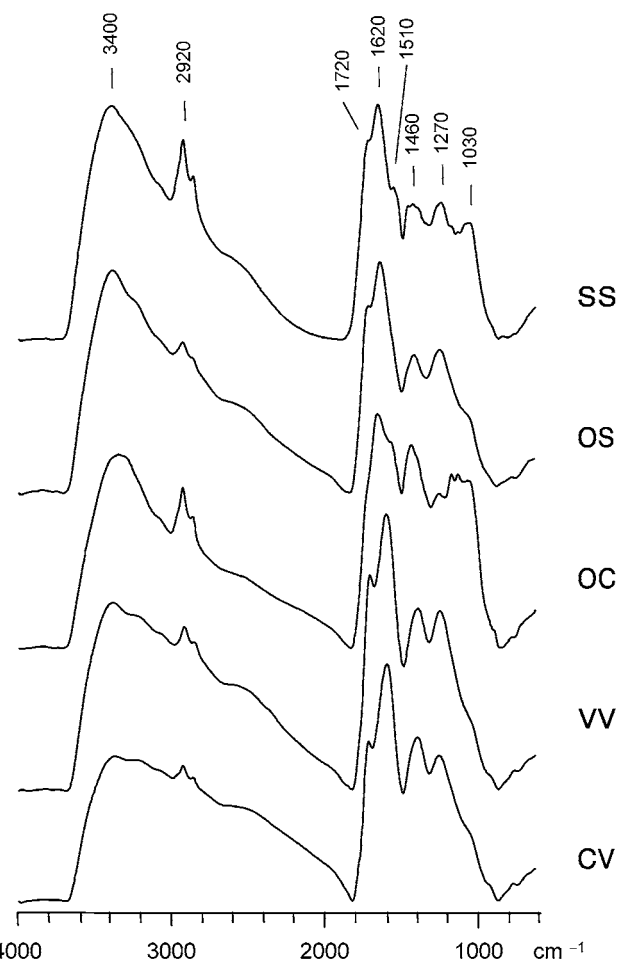

Figure 4. Fourier transform infrared spectra of humic acids from northern Botswana ecosystems. Each spectrum was obtained by digital averaging of the spectra of two samples taken from different sites. For soil labels, refer to Materials and Methods.

tion, which is frequently taken as the most valuable surrogate indicator for aromaticity and maturation of the humic matter, at least when the latter concept is defined in terms of the extent to which plant- and microbe-derived organic matter has turned into a material with a different chemical composition and structure (*41*, *42*). In particular, their very dark color was measured at half the concentration (i.e., 100 mg L⁻¹) valid for most soil humic acids (to comply with Lambert−Beer's law). These optical density values (**Table 4**) suggest a large aromaticity with a probable high concentration of stable free radicals. There was a small nonsignificant increase in the specific
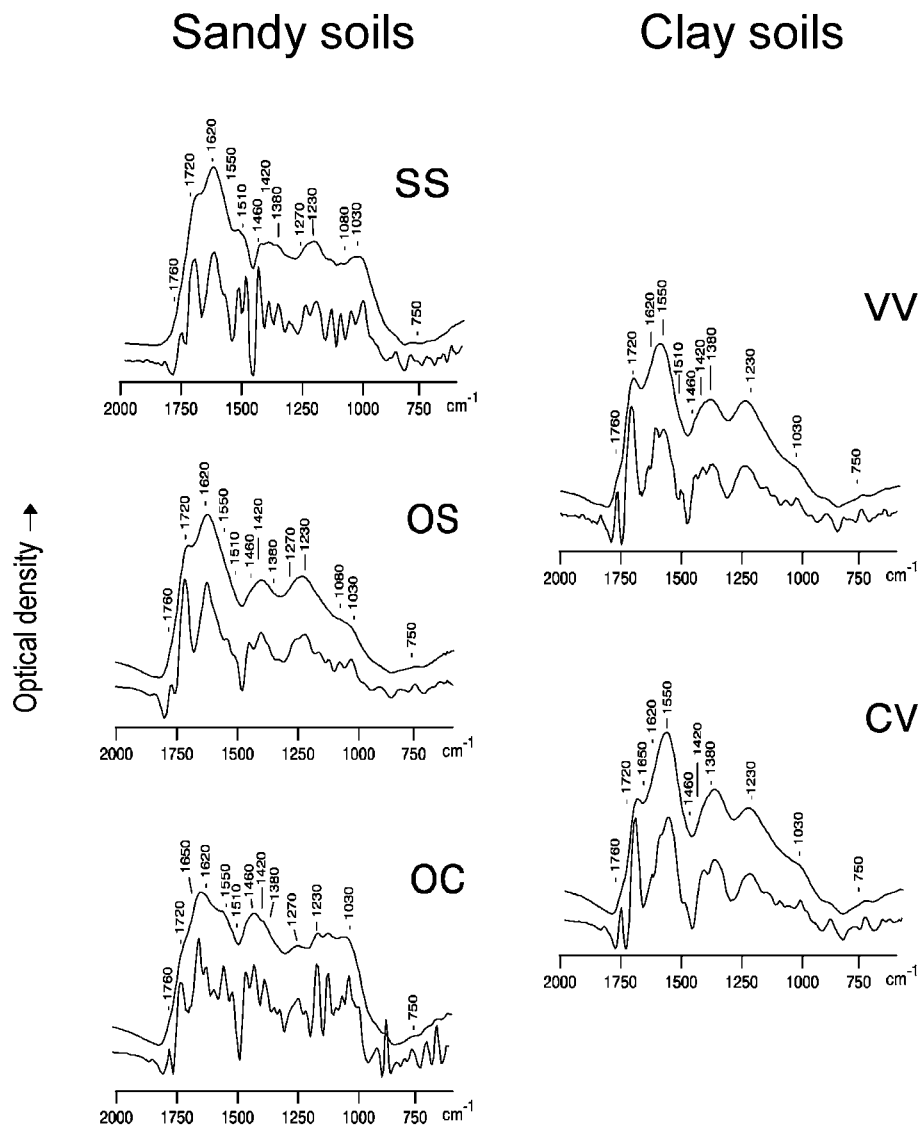
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Figure 5. Fourier transform infrared spectra and resolution-enhanced infrared spectra (bottom) representative of humic acids from northern Botswana ecosystems. Each spectrum was obtained by digital averaging of the spectra of two samples taken from different sites. For soil labels, refer to Materials and Methods.

extinction of humic acids as a result of cultivation. The E_{465} tends to be greater and the E_{465}/E_{665} ratio (*43*) lower in clayey than in sandy soils, thus indicating a deeper transformation of the humic acids in the former.

The high optical density values found in all Pandamatenga soils are also typical of fossil organic matter forms because they require the contribution of abiotic processes such as (i) the influence of intense geophysical conditions such as a high pressure in microaggregate compartments in Vertisols in an environment affected by highly contrasting climatic conditions and where eolian transport could distribute fine particles generated under these conditions; (ii) the lasting effects of fires in the past, leading to aromatization of the organic matter (*13*); and (iii) redox cycles in seasonally waterlogged ecosystems favoring the condensation of humic acids, with an effect in increasing optical densities (*19*). In particular, the effect of heating organic matter (because of the long-term exposure to intense solar irradiation or the lasting effect of fires, which may remain on the century time scale) has been frequently invoked to explain the origin and stability of most carbon forms (*4, 13, 38, 39, 44*). In an extreme situation, the above-mentioned particulate recalcitrant carbon forms included in the generic term

"black carbon" are considered to represent a significant sink of atmospheric CO_2, thus contributing to fire-induced carbon sequestration (*45, 46*). Alternative hypotheses to explain the extremely dark color of the humic acids from Pandamatenga soils were considered to have little influence in the case under study. For example, the second-derivative spectra of most humic acids formed in forest soils show recognizable valleys revealing the presence of a frequent chromophor (dihydroxyperylen-quinone) formed by several types of soil fungi. These fungi synthesize dark-colored melanins that accumulate in the humic acids under different climatic conditions (*47*) but were practically absent in all samples taken in northern Botswana, where no contribution of these pigmented fungi to the accumulation of soil organic matter was observed.

The above interpretations are in agreement with the results provided by IR spectroscopy (**Figure 5**). The IR spectra consist of very broad bands and resemble some types of fossil organic matter (e.g., oxidized carbon), showing dominant bands for aromatic structures (1510 and 1620 cm^{-1} peaks) and low-intensity bands for alkyl groups (2920 cm^{-1}). The extremely featureless shape (i.e., broad-band profile) of the IR spectra also points to an intense transformation of plant and microbial
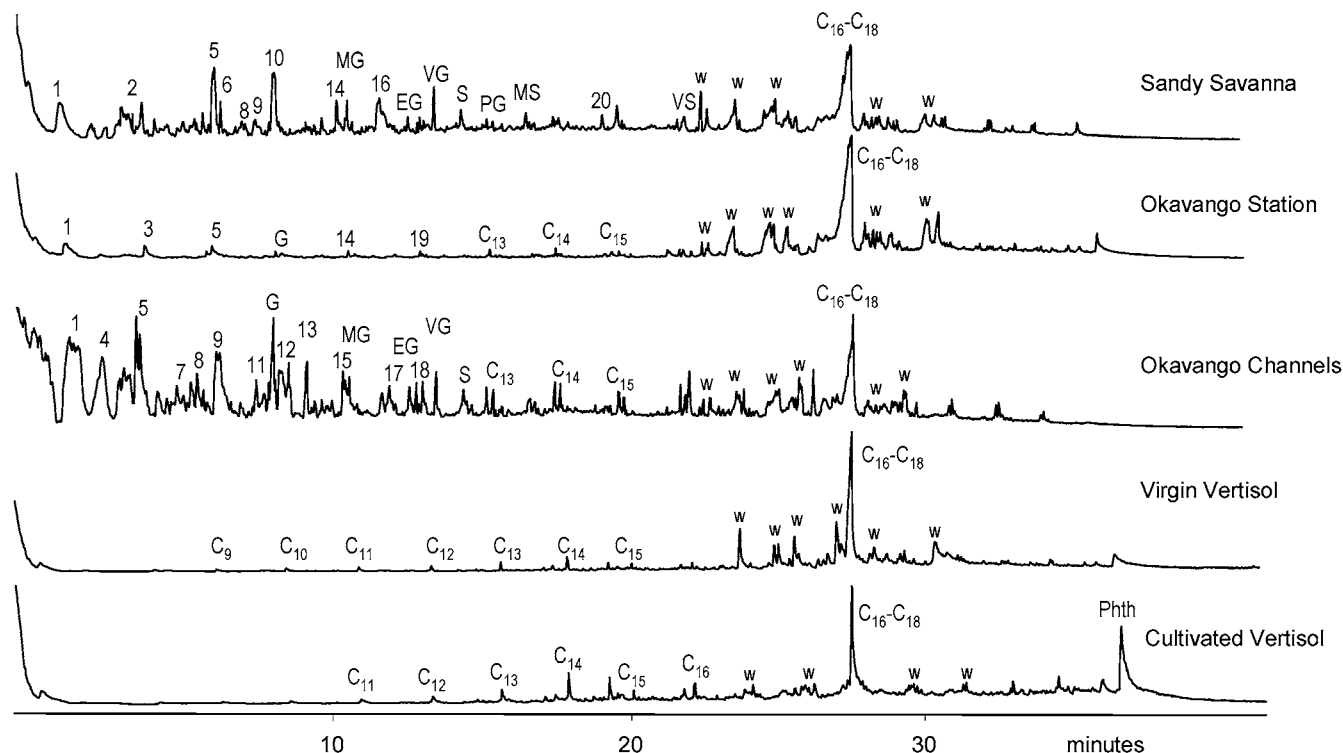
Figure 6. Chromatographic separation of the Curie-point pyrolytic compounds released by humic acids. Major compounds are labeled on the peaks: 1, methylbenzene (toluene); 2, dimethylpyrazole; 3, styrene; 4, ethylpyrrole; 5, phenol; 6, C_2-alkylbenzenes; 7, C_4-alkylpyrrole; 8, C_3-alkylbenzenes; 9, C_2-phenol; 10, C_2-benzenediol (catechol); 11, methoxybenzene; G, guaiacol; 12, methylphenols; 13, 2-methylbenzonitrile; 14, methylmethoxybenzene; 15, C_4-alkylbenzene; MG, methylguaiacol; 16, C_5-alklylbenzene; 17, C_2-indene; EG, ethylguaiacol; 18, indane; 19, methylnaphthalene; VG, vinylguaiacol; S, syringol; PG, propenylguaiacol; MS, methylsyringol (MS); 20, methylfluorene; VS, vinylsyringol (VS); 21, hexadecanoic acid octadecyl ester; w = wax esters; Phth = dialkyl phthalate.

macromolecules, which we attribute to the effect of abiotic processes, which have smoothed the original signature of macromolecules frequent in most humic acids from temperate soils.

A small but systematic difference between virgin and cultivated Vertisols was found after resolution enhancement of the spectral profiles (**Figure 6**). This difference consisted of a spectroscopic pattern similar to that of plant lignins (at 1510, 1460, 1420, and 1380 cm^{-1}), which almost completely disappeared in the cultivated plots (*48*).

The peak intensity values (**Table 4**) indicate a higher accumulation of alkyl structures in Arenosols than in Vertisols. This is suggested by the intensity of the peaks produced by alkyl stretching (2920 cm^{-1}) and alkyl bending (1460 cm^{-1}). Concerning oxygen-containing groups, the intensity of the H-bonded OH groups (3400 cm^{-1}) is much higher in the Arenosols and the 1720 cm^{-1} carboxyl peak is more responsive to the incidence of waterlogging (Arenosols) and significantly more intense in virgin than in cultivated Vertisols. In general, the minor effect of clearing and cultivation on Vertisols is probably due to the endodinamorphic character of these soils. There was no appreciable increase in aromaticity [reported after cultivation of other nonvertic soils (*49*)], which agrees with the resilience of the organic matter inferred from the quantitative patterns of the humus fractions. The Arenosols spectra in **Figure 5** might indicate that increasing soil moisture leads to selective preservation of plant and microbial macromolecules (site OC), whereas in the well-drained site there was a much lower transformation of the organic matter (site SS). Consequently, the humic acids in site OS showed the deepest transformation among the Arenosols. This is interpreted as a favorable effect of seasonal changes in soil moisture regime as suggested above.

Analysis of the humic acids by Curie-point analytical pyrolysis (**Figure 6**) confirmed some of the previous information obtained from the quantitative analysis of the humus fractions but failed to provide extensive additional information because the compound assemblages produced by thermal cleavage of these humic acids were in general relatively poor, which is tentatively interpreted as if they were to a small extent representative of the whole structure of the humic acids.

In fact, when compared with the pyrograms of humic acids from temperate soils (*50*), only traces of simple aliphatic compounds (both carbohydrate-derived and alkyl compounds such as alkanes, fatty acids, and olefins) were found. As major pyrolysis compounds all samples showed a series of wax esters derived mainly from saturated $C_{12}-C_{18}$ acids and similar long-chain alkanes, the most abundant compound being hexadecane acid octadecyl ester (*51*). In some samples, as in Vertisols, or Arenosols at the OS site, these substances were practically the only compounds released by pyrolysis. The remainder pyrolysate consisted of a small series of doublets of alkenes and α,ω-alkadienes in the C_6-C_{21} range and a carbon preference index close to unity, which could correspond to traces of cutans (*52*).

The above patterns suggest that the major pyrolysis products were only thermoevaporation products, probably minor constituents of the whole sample, but the only ones released from a condensed aromatic matrix, which should be recalcitrant against thermal degradation. Such a poor pyrolysis pattern coincided with that described for black carbons (*53*) subjected to analytical pyrolysis under the same conditions and was also found by Poirier et al. (*38*) in recalcitrant organic matter from tropical African soils.

In the case of SS and OC Arenosols, the pyrolytic patterns were more similar to those of temperate soils, but practically

all of the pyrolytic fragments corresponded to aromatic compounds with a clear origin in lignins [i.e., the typical signature methoxyphenols (*50*)] in addition to some alkylbenzenes, phenols, and catechols. This coincided with the resolution-enhanced IR spectra indicating a lower evolution of the organic matter and some accumulation of plant lignins. The pattern in the waterlogged sandy soil (OC) was substantially similar but richer in compounds including nitrogen molecules expected from the aquatic humic substances. In any case, the lack of a conspicuous series of alkanes and fatty acids, as well as of typical aromatic series characteristic for coalified materials (naphthalenes, anthracenes, fluorenes, tetralins, etc.), is probably pointing out that even in these samples most humic acid constituents remain invisible for analytical pyrolysis. In fact, the light humin fractions in these soils (not shown) display a "flat" pyrogram comparable to that of Vertisols and the OS Arenosol, indicating that a substantial amount of C in this soil is not amenable to standard thermal methods.

From the viewpoint of the environmental factors involved in the humification processes, comparison of the pyrolytic patterns could indicate that both the contrasted moisture regime prevailing in the OS soil and the mineral fraction in the Vertisols play a substantial role in the accumulation of extremely recalcitrant organic matter forms (*27*), whereas the only evidence of young organic matter surviving biodegradation processes was found in SS and OC sites. This is attributed, in the former case, to a low water-holding capacity, which limited the microbial population and the transformation of plant litter, and, in the latter case, to typical selective preservation processes prevailing in waterlogged media.

Conclusions. The results suggest that the high stability and intense transformation degree found in the humic acids in the area studied seem not to depend on the characteristics of the lithological substrate and the conditions introduced with the agricultural practices. Such a stability of the humus fractions compared to values in forest and agricultural soils under temperate climates may be due to external factors, the most relevant being probably the contrasting seasonal climate (*27*) associated with intense solar irradiation on a soil system where the accumulation of organic matter is limited by the historical effect of bush fires and heavy grazing pressure (*4*). Only a small amount of soil organic matter accumulates in these ecosystems, but these carbon and nitrogen forms represent quite stable pools. From an agrobiological viewpoint, the organic matter is preferentially retained in specific organomineral microcompartments and represents in these soils a stable constituent of the soil matrix rather than a source of plant nutrients.

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