



# Increase in stability against thermal oxidation of soil humic substances as a result of self association

P. Buurman<sup>a,\*</sup>, B. van Lagen<sup>a</sup>, A. Piccolo<sup>b</sup>

<sup>a</sup>Laboratory of Soil Science and Geology, Wageningen Agricultural University, PO Box 37, 6700 AA Wageningen, The Netherlands

<sup>b</sup>Dipartimento di Scienze Chimico-Agrarie, Università di Napoli "Federico II", Via Università 100, 80055 Portici, Italy

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## Abstract

The stability to thermal oxidation of soil humic extracts saturated with H, Na, Ca, or Al, was followed after treatment with relatively polar organic compounds, such as methanol, formic acid, and acetic acid. While thermal characteristics of H-humates did not change upon addition of the polar molecules, thermal decomposition (oxidation) of Na-humates was shifted to much higher temperatures (750–830 °C) than control. Substantially less dramatic was the effect on Ca-humates, whereas hardly any alteration was observed when polar organic compounds were added to Al-humates. These results can be explained by considering the forces that hold humic molecules together. Humic components are strongly bound to each other by hydrogen bonding in H-humates and by electrostatic bridges in Ca- and Al-humates. These binding forces were not overcome by the simple addition of polar organic molecules, and their stability remained generally unchanged. In Na-humates, associations of humic molecules are held together only by non-specific hydrophobic interactions. Our results showed that wetting the relatively more flexible Na-humates with organic solvents slightly less polar than water caused a significant increase in thermal stability. Because most polysaccharide-C has largely disappeared at 400 °C, this thermal behaviour can be explained by the rearrangement of largely hydrophobic humic components in methanol, leading to an increase in association energy. The intensity and reversibility of thermal stabilization indicate that association occurred among relatively small molecules rather than among macromolecules. Also Na-saturated humus of bulk samples showed an increase in stability against oxidation upon addition of methanol. These findings suggest that, counter-ions and amphiphilic organic compounds may affect organic matter stability also in natural soils. © 2002 Elsevier Science Ltd. All rights reserved.

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## 1. Introduction

In the past 30 years, the thermal properties of soil humic substances were largely studied to obtain information on their chemical nature (Turner and Schnitzer, 1962; Scharpenseel et al., 1964; Schnitzer and Hoffman, 1967; Dupuis et al., 1970; Jambu et al., 1972; Campanella et al., 1990; Maizenberg et al., 1991; Leinweber and Schulten, 1992; Leinweber et al., 1996). Such studies were carried out on extracted and reprotonated organic fractions, sometimes saturated with specific metal

cations. They invariably indicated that the thermograms of humic substances were strongly dependent on pre-treatment and saturating cations, although there was no good explanation for such dependence. Buurman et al. (1992) established a relation between thermal stability and Al-saturation of untreated podzol-B humus, and Buurman and Aran (1997) suggested a relation between thermal stability and microbial decay in podzol humus.

No significant correlation was ever found between the thermal properties and composition of soil organic matter. This difficulty in relating thermal stability of soil humic substances to their chemical nature is probably due to chemical heterogeneity and to our still limited knowledge of conformational behaviour.

Recent results have indicated that humic substances, rather than being macromolecules, may be constituted

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\* Corresponding author. Fax: +31-317-482419.

E-mail address: peter.buurman@bodeco.beng.wau.nl (P. Buurman).

of smaller molecules self-assembling in supramolecular structures through weak forces such as hydrogen and hydrophobic bonds (Piccolo et al., 1996, 1999; Conte and Piccolo, 1999; Piccolo and Conte, 2000; Conte et al., in press). These studies showed that protonated humic associations in aqueous solution are reversibly disrupted into smaller associations by small additions of methanol and different organic acids, which alter the conformational arrangement between humic hydrophilic and hydrophobic domains.

It can be envisaged that as treatments with organic acids modify the conformational behavior of soil humic substances, they should also alter the energy content of the humic arrangements and, hence, the thermal stability of such arrangements. Such changes would be similar to the differences in energy content of conformational isomers of a humic building block observed by Sein et al. (1997). Changes in thermal stability, i.e. the threshold of decay by oxidation, would probably also affect decomposability by microbes, and thus the stability of humus in the soil environment. We studied the effect of changing conformational behaviour, due to the addition of relatively polar molecules, on extracted and natural humus with different cation saturation.

## 2. Materials and methods

### 2.1. Soil samples, isolation and characterization of humic substances

The H and B-horizons of an incipient podzol under Scots pine with billberry undergrowth (Nierop and Buurman, 1998; Nierop et al., 1999) on inland dunes in the Netherlands were sampled. NaOH-soluble material was separated, acidified to pH 1, dialyzed to pH 7, and freeze-dried. This resulted in protonated humic fractions H and B.

C and N contents were measured with a Fisons EA1108 elemental analyzer. The overall chemical composition of the humic extracts was assessed by cross-polarization magic angle spinning carbon-13 nuclear magnetic resonance (CPMAS <sup>13</sup>C-NMR) spectroscopy. Spectra were obtained on a Bruker AMX400 operating at 100.625 Mhz on the carbon-13. The rotor spinning rate was set at 4500 Hz. A recycle time of 1 s and an acquisition time of 13 ms were used. All the experiments were conducted with a variable contact time (VCT) pulse sequence in order to find the optimum contact time (OCT) for each sample and to minimize the error on the evaluation of the peak areas (Conte et al., 1997). OCT ranged from 0.8 to 1 ms. A line broadening of 50 Hz was used to transform the free induction decay (FID). The area in the 110–140 ppm region was corrected for that of the spinning side band of the signal of the carboxylic groups. The correction was made by measuring the area

of the side band in the 190–230 ppm interval and by subtracting twice this area from that of the 110–140 ppm region.

### 2.2. Na-, Ca-, and Al-forms of humic extracts and untreated samples

Protonated and dialysed H and B extracts were redissolved in 0.1 M NaOH, saturated Ca(OH)<sub>2</sub>, or 0.1 M AlCl<sub>3</sub> to obtain their Na-, Ca-, and Al-saturated form, respectively. The solutions were shaken end-over-end for 24 h and dialyzed against demineralised water to pH=7 or until free of chloride. After dialysis, the extracts were freeze-dried and the ash contents determined by thermogravimetric analysis (TGA) to 1000 °C with a heating rate of 10 °C /min and an air flow of 50 ml/min, equal to the experimental circumstances of differential thermal analysis (DTA, see below).

Original, non-extracted samples from the H-horizon were also saturated with different cations as described above.

### 2.3. Addition of methanol and acids

Freeze-dried humates were wetted in excess pure methanol, formic acid, or acetic acid, and were left to air dry. Thermal analysis was performed four hours after contact for the methanol-treated samples, whereas samples treated with formic or acetic acid were air-dry after about one week. To check stability over time of the changes induced by treatments, additional thermal analyses were done after two to three weeks after the initial wetting. In some methanol-treated samples, the changes in thermal stability were followed during the first hours of contact. In an attempt to reverse the effect of methanol addition on Na-humates (which was the strongest effect observed), the Na-saturated B-extract was subjected to the following three treatments after the addition of methanol:

- (a) drying at 50 °C for 6 days;
- (b) drying at 50 °C for 6 days, followed by addition of excess water, sonication, and drying at 50 °C for 4 days;
- (c) drying at 50 °C for 6 days, followed by addition of water, sonication and freeze-drying.

### 2.4. Thermal analysis

TGA and DTA of H and B humic samples with different cation saturation were conducted with Thermal Analyst TA 2000 instrument in combination with a DuPont 951 Thermogravimetric Analyzer and a 1200 °C DTA cell. Sample weights were 1–4 mg for all samples, but 6–10 mg for Na-saturated extracts to which methanol

had been added 1–4 h before measurement. The heating rate was 10 °C/min, under a 50 ml/min flow of air. The temperature scale was calibrated using the melting point of Indium (156.6 °C), the alpha-beta conversion of quartz (573 °C), and the melting point of potassium sulfate (1069 °C). The thermobalance was calibrated with standard weights and by the weight loss curve of calcium oxalate monohydrate.

### 3. Results

#### 3.1. Characteristics of humic extracts

The elemental analysis and the carbon distribution in the various chemical structures are reported in Table 1 for the protonated form of the H and B extracts. NMR composition of both samples is very similar. The C content of the B extract is slightly lower because of its higher ash content. Ash contents were 4.2 and 9.8% in H-saturated fractions of the H and B horizon, respectively (see Tables 2 and 3).

#### 3.2. Thermal stability of humic acids and salts

This discussion concerns the extracted material, unless otherwise mentioned. The DTA traces of the H and B extracts in their H-, Na-, Ca-, and Al-saturated forms are shown in Fig. 1a and b, the TGA traces of the same samples in Fig. 2a and b. Thermogravimetric analyses (Fig. 2) are given to illustrate that each exothermic reaction is associated with weight loss. The temperatures of weight losses observed by thermogravimetry and the corresponding temperatures of maximum weight loss (the first derivative of the gravimetric curve) are reported in Tables 2 and 3. The weight loss between room temperature and 105 °C is an indication of either moisture or excess ('free') organic reagent (methanol, formic acid, acetic acid). Temperature indications in this text are maxima of DTA measurements. The values for the maximum derivative of the weight loss curves as used in Tables 2 and 3 may be somewhat different.

Our results indicate that there was a profound alteration of the thermal behaviour of the humic material when the protons were substituted with mono-, di-, and tri-valent cations. In protonated H and B material, the thermogram showed three main exotherms (Fig. 1a and b). The first exotherm occurred around 310–330 °C, and the other two at 484 and 503 °C for the H sample and at lower temperatures (432 and 484 °C) for the B material.

Saturation with Na of the H horizon extract (Figs. 1a and 2a and Table 2) shifted the first and second exothermic peaks to considerably lower temperatures, while a third exotherm appeared at higher temperatures (692 °C). A similar shift to both lower and higher oxidation temperatures was observed for the B humic material (Figs. 1b and 2b, and Table 3).

Compared to H-saturated samples, Ca-saturation of extracts of both horizons showed a shift to lower temperatures of the most intense exotherm (484 → 380 °C in H; 432 → 380 °C in B), while the low-temperature exotherm remained essentially unchanged. In both samples, small exotherms appeared around 500 °C (492 in H, 521 in B) and between 600 and 700 °C (630 in H, 658 in B).

With respect to H-saturated humus, Al saturation resulted in smaller size of the first exotherm and increase of the second. The first was around 360 °C, a slightly higher temperature than in the protonated samples, and the second, more intense, was at 430–470 °C, close to that of the protonated samples but less sharp. In both the H- and B-sample, the exotherm around 500 °C disappeared upon saturation with Al.

#### 3.3. Modification of thermal stability by methanol and organic acids

In protonated humus (H-humates), addition of methanol, formic acid, or acetic acid did not result in changes in thermal properties and, hence, no data related to H-saturated samples are reported.

Fig. 3a and b show how wetting with methanol substantially modified the thermal behaviour of the Na-salts of both H and B humus. While the first exothermic peak (around 280–290 °C) still accounted for 30 wt.% decrease in the dry samples, the largest weight loss accompanied the appearance of a surprisingly sharp and

Table 1  
Elemental analyses (mass%) and relative <sup>13</sup>C distribution (%) of protonated H and B samples, as calculated in resonance intervals (ppm) from CPMAS-NMR spectra

Sample	C (%)	N (%)	C/N	<sup>13</sup> C chemical shift interval (ppm)				
				0–40 Alkyl	40–60 C–N	60–110 C–O	110–160 Aromatic	160–190 Carbonyl
H	49.7	1.87	26.5	26	39	18	11	6
B	42.3	1.87	22.6	26	40	17	12	5
H-400 °C				16		9	50	25

Table 2

Temperatures (°C) of exotherms and relative mass losses (mass%) in thermogravimetric analyses (TGA) of H-horizon extracts and their salts<sup>a</sup>

Sample <sup>b</sup>	ML <sup>c</sup>	Temperature ranges										Ash 1000 °C
		105–350 °C		350–450 °C		450–550 °C		550–700 °C		> 700 °C		
		T	WL <sup>d</sup>	T	WL	T	WL	T	WL	T	WL	
<i>H</i>												
H-sat.	8.0	319	30.7	–	15.2	520	39.1	–	8.9	–	1.4	4.7
<i>Na-sat</i>												
Control	13.9	285	31.4	–	12.7	428	7.2	704	12.5	–	26.9	9.3
MeOH	35.2	282	30.7	–	–	3.7	–	–	–	807	53.9	11.7
HFor	7.5	288	43.1	–	–	8.9	–	–	–	822	40.4	7.7
HAc	25.4	282	30.2	–	–	481	19.5	–	–	756	31.8	11.5
<i>Ca-sat</i>												
Control	17.3	309	27.9	365	31.0	484	11.9	663	9.4	–	0.6	19.2
MeOH	40.0	–	–	362	57.3	477	23.7	–	–	–	7.5	12.5
HAc	25.1	322	37.5	433	30.8	510	8.1	623	10.2	–	2.2	11.2
<i>Al-sat</i>												
Control	2.9	–	28.7	381	33.2	467	27.4	–	1.0	–	2.4	9.7
MeOH	64.5	–	–	344	39.3	450	43.8	–	2.5	–	–	14.3
HFor	84.6	295	67.6	–	–	492	20.6	–	3.2	–	–	8.5
HAc	19.6	–	–	338	45.7	461	48.3	–	1.8	–	–	7.9

<sup>a</sup> Temperature readings are from TGA and may differ slightly from DTA curves; dashes indicate the absence of a clear exotherm.

<sup>b</sup> Control: humic sample without any addition; MeOH: methanol; HFor: formic acid; HAc: acetic acid.

<sup>c</sup> ML: moisture loss (mass%) (20–105 °C) refers to the total weight of sample at 20 °C.

<sup>d</sup> WL: weight loss belonging to the respective exotherm. Mass% of dry sample (105 °C).

Table 3

Temperatures (°C) of exotherms and relative mass losses (mass%) in thermogravimetric analyses (TGA) of B horizon extracts and their salts<sup>a</sup>

Sample <sup>b</sup>	ML <sup>c</sup>	Temperature ranges										Ash 1000 °C
		105–350 °C		350–450 °C		450–550 °C		550–700 °C		> 700 °C		
		T	WL <sup>d</sup>	T	WL	T	WL	T	WL	T	WL	
<i>B</i>												
H-sat	11.3	312	34.4	453	26.2	502	23.0	–	6.0	–	0.8	9.7
<i>Na-sat</i>												
Control	14.2	270	30.4	–	10.4	–	8.5	665	17.5	–	13.2	20.0
MeOH	31.1	280	31.0	–	–	–	12.7	–	–	830	41.8	14.5
HFor	22.7	272	40.3	–	–	–	11.7	–	–	826	42.6	5.4
HAc	17.5	272	28.1	–	–	–	6.8	–	–	845	54.3	10.8
<i>Ca-sat</i>												
Control	17.3	306	32.2	376	25.8	530	4.6	679	15.5	–	1.3	20.7
MeOH	14.0	316	25.5	379	24.9	525	17.5	644	8.4	–	3.4	20.8
HAc	58.9	290	31.4	420	8.3	504	28.1	643	10.0	–	3.3	17.0
<i>Al-sat</i>												
Control	12.9	342	32.4	430	40.0	–	11.5	–	0.7	–	4.0	11.5
MeOH	19.1	–	–	354	34.5	418	45.1	–	3.0	–	–	17.4
HFor	30.6	294	31.6	–	–	423	45.0	–	4.4	–	–	19.0
HAc	11.0	287	44.8	–	–	428	37.3	–	2.5	–	–	15.4

<sup>a</sup> Temperature readings from TGA may diverge slightly from DTA readings. Dashes indicate the absence of clear exotherm in DTA.

<sup>b</sup> Control: humic sample without any addition; MeOH: methanol; HFor: formic acid; HAc: acetic acid.

<sup>c</sup> ML: moisture loss (mass%) (20–105 °C) refers to the total weight of sample at 20 °C.

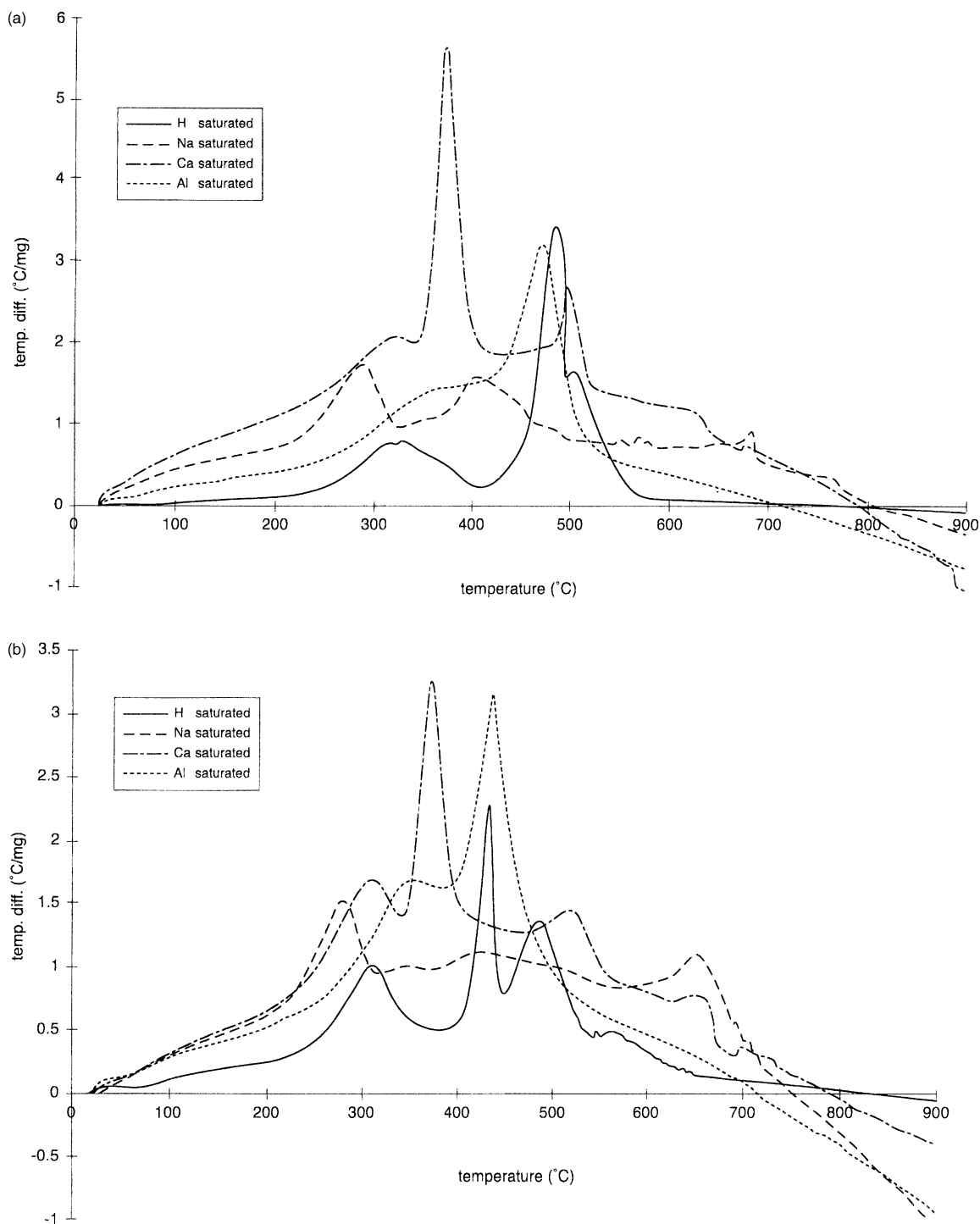


Fig. 1. DTA traces of protonated, Na, Ca, and Al-saturated H- and B-horizon extracts. 1a: H-horizon; 1b: B-horizon.

intense exothermic peak at 807 °C and at 830 °C for both sodium humates (Tables 2 and 3). Thermal analyses carried out during the first hours of contact with methanol show that this change in thermal behaviour is

completed within 5 h. Incomplete, but significant change was already obtained with relatively small additions of methanol, e.g. 3  $\mu$ l of methanol on 2 mg of sample. Experiments with Na-saturated H-horizon humus (data

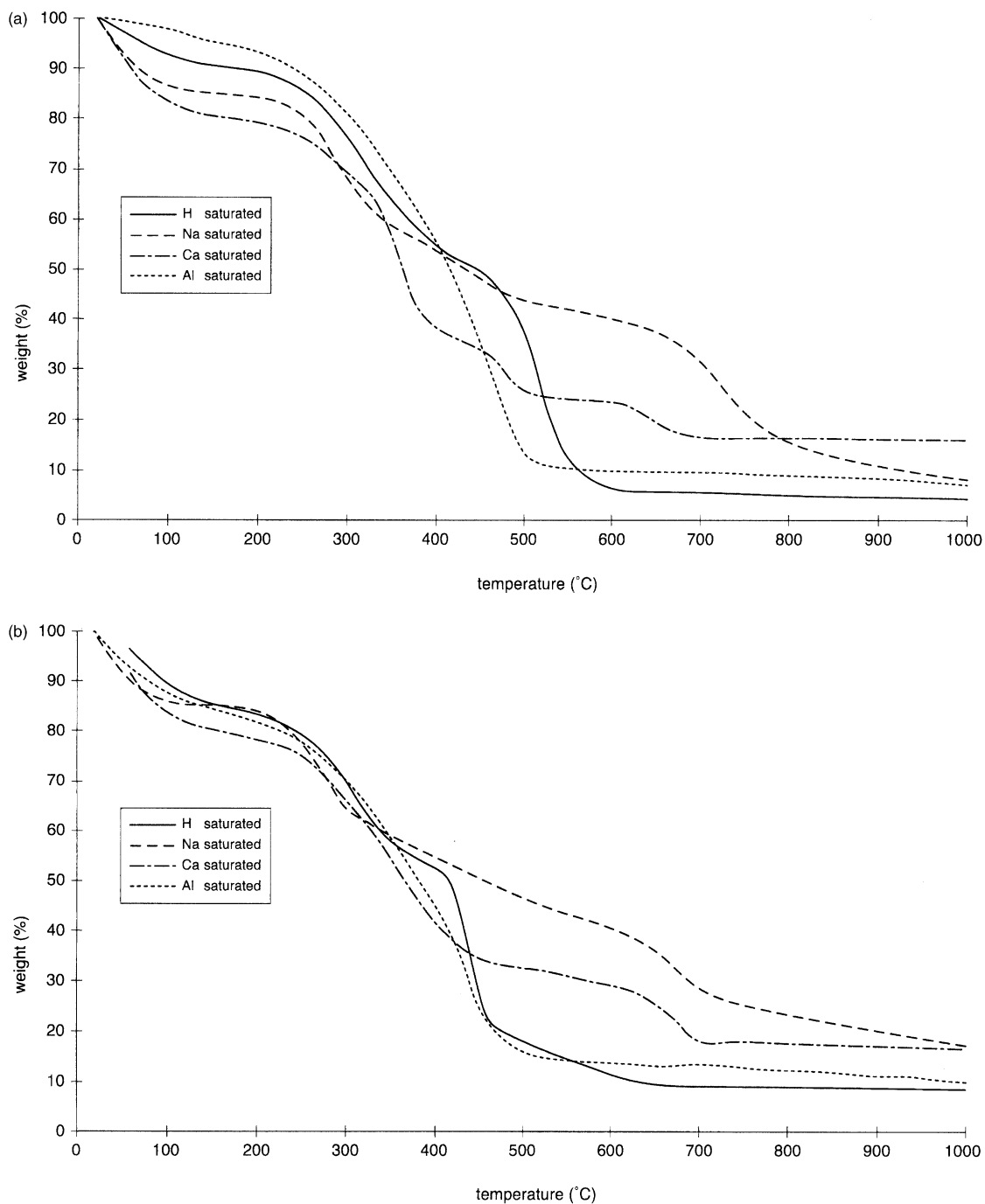


Fig. 2. TGA traces of protonated, Na, Ca, and Al-saturated H- and B-horizon extracts. 2a: H-horizon; 2b: B-horizon.

not reported here) suggest that the bound methanol (and remaining water) amounts to about 11% by weight with respect to the oven-dried fraction (105 °C).

Addition of formic acid to Na-saturated H- or B-horizon humus also caused high-temperature exotherms to appear, but to a lesser extent than methanol. A

broadening of the low-temperature exotherm was observed, but the weight loss in this region was still substantial (>40%). A considerable fraction was stabilized at higher temperatures, since the weight loss above 700 °C was about 40 and 42% in H and B samples, respectively.

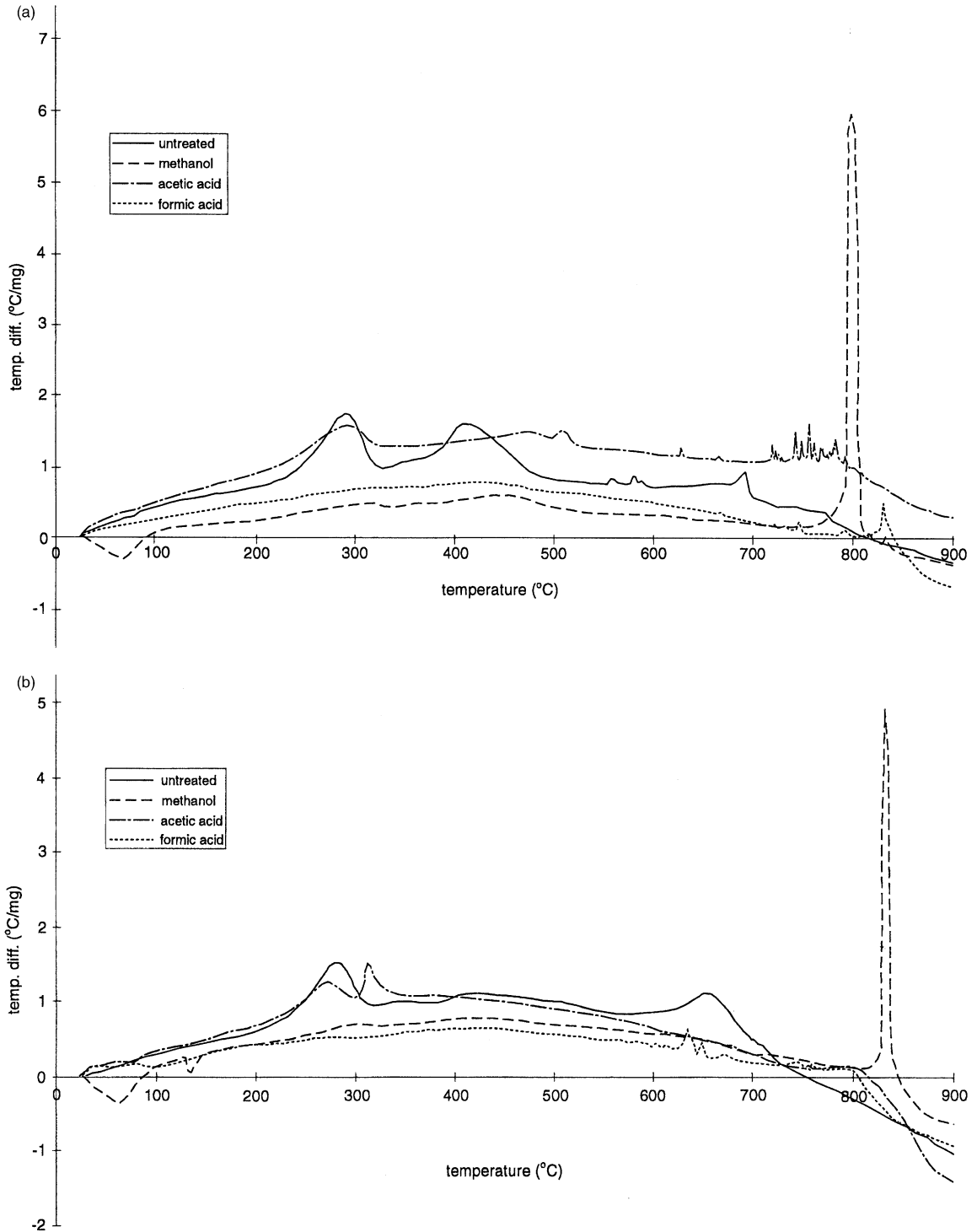


Fig. 3. DTA traces of Na-saturated H- and B-horizon extracts upon addition of methanol, formic acid, and acetic acid. 3a: H-horizon; 3b: B-horizon.

After addition of acetic acid to Na-saturated H-horizon humus, the exotherms in the 250–350 °C and 350–450 °C ranges were still present, but a significant series of exotherms appeared at higher temperatures. The weight loss above 700 °C amounted to some 32%. Acetic acid also caused a clear high-temperature exotherm in B-horizon humus, related to a weight loss of 54%.

A different thermal response was given by the Ca-salts of both H and B materials after wetting with methanol and acetic acid. Methanol caused a sharpening of the exothermic peaks of the H sample calcium-humate but did not shift their temperatures significantly (Fig. 4a). Even less effective was the methanol treatment on the thermal profile of the Ca-saturated B humus. The exothermic peaks remained essentially similar in shape and temperature (Fig. 4b and Table 3). The addition of acetic acid produced a more significant alteration of the thermal profiles of the Ca-humates than that of methanol. The first small exotherm at around 300 °C remained unchanged in both H and B calcium humates, whereas in the H sample the exotherm peak between 350 and 400 °C disappeared and a group of two to three small peaks appeared between 450 and 550 °C (Fig. 4a). This was especially prominent in the DTA profile of the Ca-saturated B material (Fig. 4b), where the high-temperature exotherm became much sharper and more intense than the broader exotherms noted in both the untreated and methanol-treated samples.

The Al-saturated H and B material appeared to be less sensitive to wetting with methanol and organic acids. The shape of the thermal profile for the H material remained essentially unchanged upon the different additions (Fig. 5a), and temperature shifts of the exotherms were minimal (Table 2). Similar behaviour was exhibited by the thermal profiles of the Al-humates of sample B, with the exception of an intense sharpening of the main peak at around 430 °C produced by the formic acid addition (Fig. 5b).

### 3.4. Experiments on bulk soil samples

Wetting with solvents of bulk soil samples saturated with single cations gave less spectacular changes in stability than humic extracts. However, thermal stability of the Na-saturated bulk samples was somewhat increased by addition of methanol (Fig. 6). In Ca-saturated samples, addition of methanol resulted in a better definition of the exotherms, and the appearance of an exotherm above 900 °C. Formic acid and acetic acid resulted in better definition of exotherms alone. The Al-saturated samples showed no effects upon solvent addition.

## 4. Discussion

Variation in exotherm temperatures in humic salts and complexes in relation to the nature of saturating

cations has been described before. Turner and Schnitzer (1962) reported on the thermal decomposition of protonated podzol humus. Schnitzer et al. (1964) noted that saturation with Na increased the temperature at which organic matter is oxidized, whereas treatment with  $\text{Al}^{3+}$  and  $\text{Fe}^{3+}$  decreased it. Buurman et al. (1992) described the increase of decomposition temperatures with the  $\text{Al}/(\text{Al} + \text{Fe})$  ratio in untreated podzol-B bulk samples. Schnitzer and Hoffman (1967) observed that the temperature of the major exothermic peak of an untreated fulvic acid was shifted to higher temperatures when the fulvic acid had reacted with monovalent cations, but to lower temperatures when fulvic acid was complexed with di- and tri-valent metal ions. Dupuis (1971) and Jambu and Dupuis (1975) studied the effect of the type of Fe- and Al- complexes on thermal decomposition of podzol humus. High residual weights remaining in some cases after heating as high as 1200 °C were explained by the formation of inorganic carbonates (e.g. Jambu and Dupuis, 1975; Schnitzer and Hoffman, 1967). Carbonates, however, decompose to oxides and  $\text{CO}_2$  at temperatures well below 1200 °C (calcite 900–1000 °C, Na-carbonates <200 °C; Fe-carbonate 460 °C; Mackenzie, 1970; Blazek, 1973), so that a residue at such high temperatures may (partially) consist of an oxide, but not of a carbonate. Decomposition of carbonates at high temperature would cause an endothermic reaction and not an exotherm. Moreover, the fact that the reorganization took some time to be completed, and that the high temperature exotherm grew at the expense of low-temperature exotherms during the first few hours of contact, suggests an organic association rather than an inorganic phase. Large variations in ash content probably result from stabilization of the organic matter to still higher temperatures.

Our results appear to support previous findings which suggested that apparently high-molecular weight humic substances are in fact self-associations of relatively small molecules, and that their conformational structure is stabilized by weak bonds, such as hydrogen and hydrophobic bonds, that hold the different molecules together (Piccolo et al., 1996, 1999; Conte and Piccolo, 1999; Piccolo and Conte, 2000). Within humic associations, the heterogeneity of the small molecules gives rise to hydrophilic domains near to hydrophobic domains (Piccolo and Conte, 1999). This can explain both the behaviour of the untreated humates and the effect of additions. In H- and Al-humates, energy-rich electrostatic bonding disrupts hydrophobic associations, which are less disturbed in the Ca- and Na-humates. The hydrophobic associations cause a higher thermal stability for part of the Ca- and Na- associated organic matter, while this is not the case in Al- and H-saturated material.

The lack of thermal effect of additions of methanol, formic acid, and acetic acid to the protonated form of



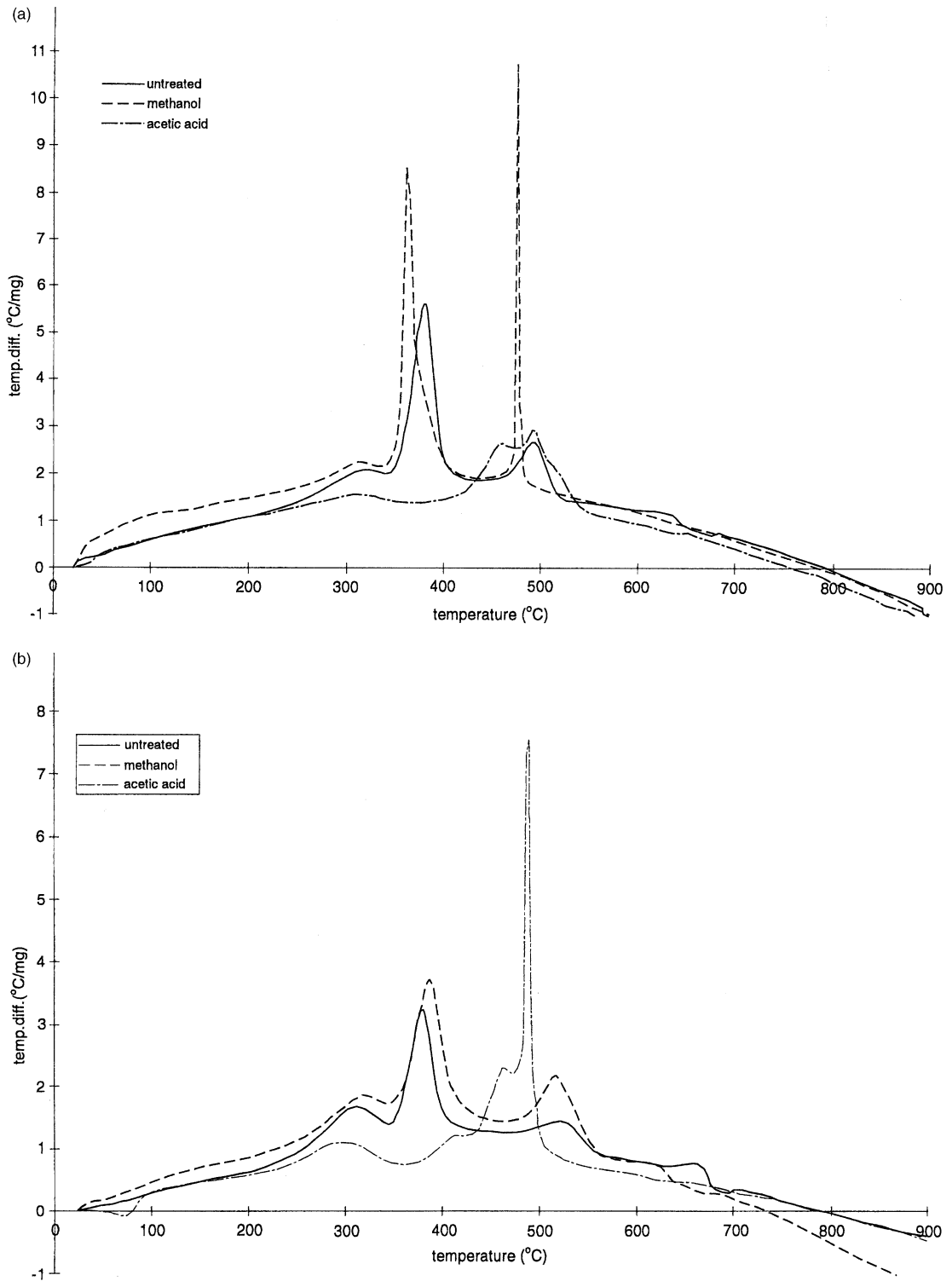


Fig. 4. Changes in DTA traces of Ca-saturated H- and B-horizon extracts upon addition of methanol and acetic acid. 4a: H-horizon; 4b: B-horizon.

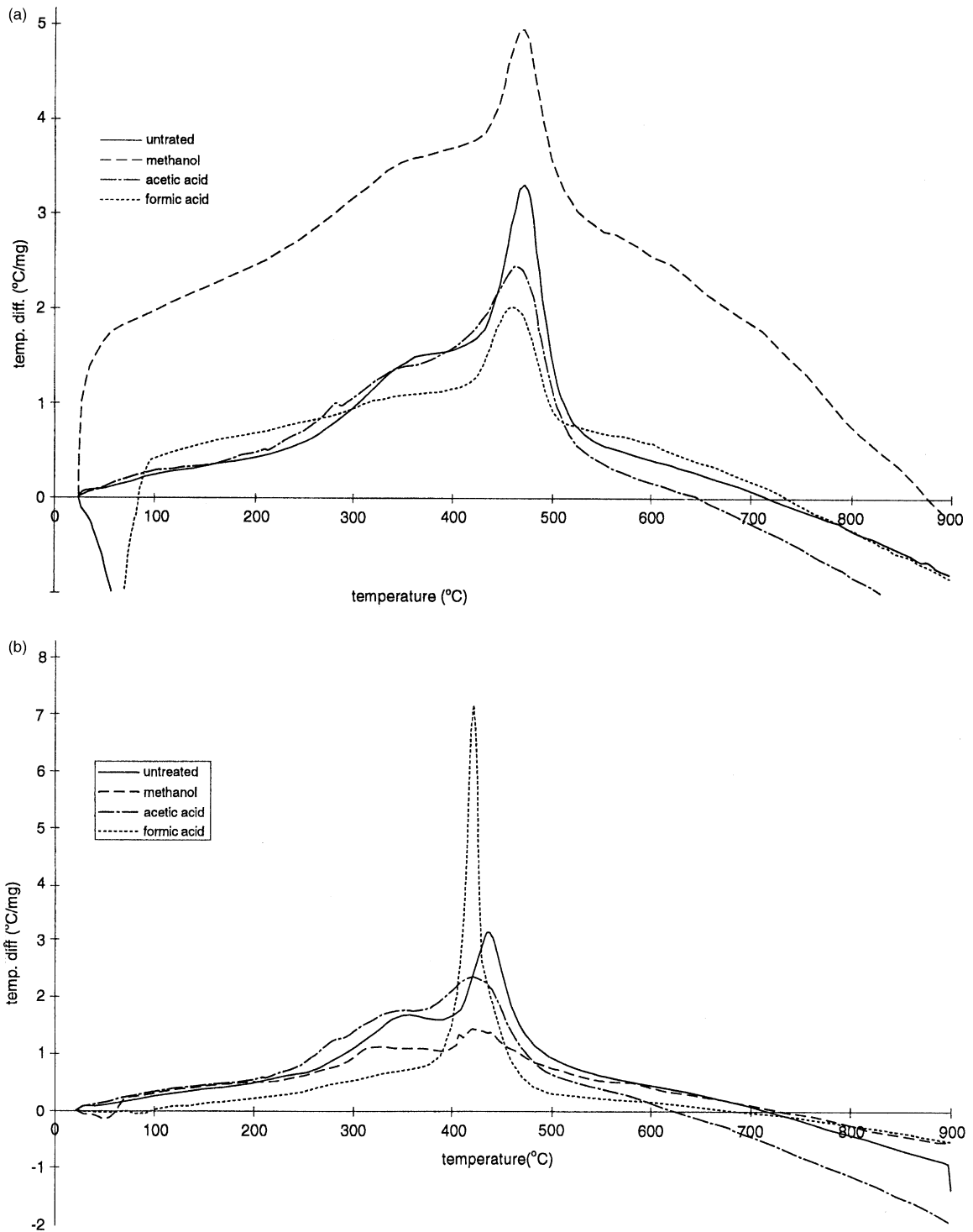


Fig. 5. Changes in DTA traces of Al-saturated H- and B-horizon extracts upon addition of methanol, formic acid, and acetic acid. 5a: H-horizon; 5b: B-horizon.

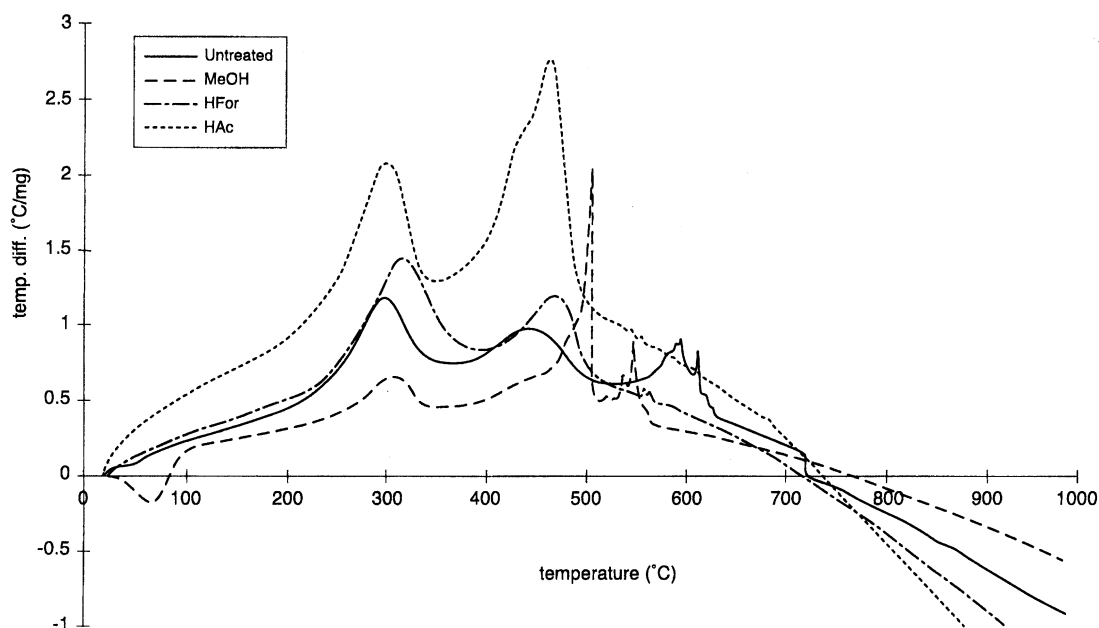


Fig. 6. Changes in DTA traces of untreated Na-saturated H-horizon bulk sample upon addition of methanol, formic acid, and acetic acid.

the two humic extracts is attributed to the relatively strong intermolecular hydrogen bonds, which form rigid conformations and inhibit molecular motion. The molecular arrangements cannot thus be significantly modified by the addition of either methanol or organic acids and the fixed conformation results in the unchanged thermal stability. The effect of intermolecular hydrogen bonds on thermal stability of humic substances has been indicated previously (Schnitzer and Skinner, 1964; Jambu et al., 1972).

The conversion of the protonated form of the humic material into their Na-salts disrupts most of the hydrogen bonds between humic molecules. The conformational structure is hence rearranged, and its stability can only be ensured by hydrophobic attractive forces between apolar humic constituents (Conte and Piccolo, 1999; Piccolo and Conte, 2000). Conte et al. (in press) showed that the apparent high molecular sizes of Na-humates is to be attributed mainly to aliphatic chains strongly associated by weak hydrophobic forces. The conformational reorganization occurring in Na-saturated humic matter also changes its thermal stability with respect to the protonated form (Fig. 1a and b). The new DTA trace reflects the thermal stability of groups of components with different capacities to establish hydrophobic interactions. The most hydrophilic components, which are unable to form multiple intermolecular hydrophobic bonds, are separated from the more apolar constituents during conformational rearrangement and thus show a definitely different thermal stability. The molecular associations of the former are

much weaker than the latter and undergo combustion at low temperatures. Conversely, the lipophilic components are more tightly bound together because of the phenomenon known as “hydrophobic effect” (Tanford, 1991; Schwarzenbach et al., 1993) and a higher energy input becomes necessary to overcome the force of their association and cause combustion. The direct relationship between carbon content and combustion temperature of a compound is well known (van Krevelen et al., 1951). It has been shown that oxidized components of forest bark and litter such as polysaccharides are thermally degraded at around 325–350 °C, whereas long-chain aliphatic constituents such as cutin and suberin are burnt only above 400 °C (Rogers et al., 1986). A similar relationship between thermal stability and molecular composition was found by Campanella et al. (1990) for humic substances obtained by different extractants. They reported infrared spectra of humic samples heated at 1000 °C, which show that some aliphatic material remained unoxidized while most of the residue consisted of silicates.

When sodium humates are wetted by methanol, the humic molecules, which have a relative mobility, rearrange their association in the methanol medium. This was readily observed in a petri dish: both with methanol and with formic acid, the fluffy freeze-dried Na-humate changes into a resin-like substance that cannot be scratched by a finger nail, as soon as the excess additive is evaporated. The relatively less polar character of methanol in comparison to water induces the humic molecules to abandon the arrangement adopted in water

or during the freeze-drying process, and assume the most stable spatial conformation in methanol, that is achieved by an enhanced separation between hydrophilic and hydrophobic humic components. Hydrophilic molecules or residual water molecules are moved into the methanol phase by hydrogen bond formation with methanol, whereas apolar molecules are effectively repelled, thereby increasing the number and strength of their intermolecular hydrophobic interaction in less hydrated humic aggregates. Tanford (1991) reports that a polar organic solvent has a structural organization that may resist the intrusion of apolar solute molecules, while it favours the solubility of polar molecules and hydration water molecules. The result is a tighter association of humic molecules, even more strongly bound by multiple non-specific bonds. Because of the high flexibility of the sodium humates, the conformational rearrangement is achieved rapidly (within 4 h). The large weight loss associated to the exotherm above 800 °C indicates that a significant part of the organic matter in the Na-humates is more strongly associated by wetting with methanol.

The relative mobility of humic molecules can also explain the very different effect observed when methanol is added to Ca- and Al-humates (Figs. 4 and 5). Our results suggest that the charge of divalent calcium is neutralized by the dissociated acidic functions of different humic molecules. The formation of intermolecular salts with calcium limits the molecular mobility in methanol. This prevents the conformational rearrangement shown by humic salts with monovalent sodium. Calcium bridging between humic molecules is stronger than both hydrogen and van der Waals bonding and generally stabilizes the humic conformation against a rearrangement driven by weak forces. Nevertheless, methanol wetting still altered the association between hydrophilic and hydrophobic components, possibly affecting the mobility of the humic molecules which are not involved in complexes with metals, and forced their rearrangement into thermally and chemically more homogeneous fractions (see the two sharp peaks in Fig. 4a). However, the increase in combustion temperature shown by Ca-humates was far less substantial than in Na-humates, and thermal changes still occurred after two weeks of contact with the solvents, thereby showing that the conformational rearrangement was much slower than in Na-humates.

Even less molecular mobility is allowed to humic molecules when they are saturated with Al. This trivalent cation is able to coordinate up to three different humic molecules, thereby enhancing the resulting molecular size of the humic aggregate (Yates and von Wandruszka, 2000). With  $Al^{3+}$ , the multiple binding possibilities that are available to humic molecules with more than one acidic function are likely to lead to much larger intermolecular aggregates. Therefore, methanol

cannot have any effect on the rigid conformational structure of Al-humates and, consequently, only minor changes were observed in the DTA profiles, even after 7–15 days of contact with the solvent (Fig. 5a and b).

Weight losses and exothermal reactions at temperatures below 600 °C indicate that not all organic fractions participate in formation of thermally stable humic associations, even if they do rearrange. Methanol-treated Na-humates heated to 400 °C exhibited a strong change in the NMR spectrum. The organic fraction still present at this temperature was strongly aromatic and had lost all its polysaccharides (Table 1, H-horizon). This indicates that apolar moieties predominate in the thermally stabilized fraction.

The lesser mobility of organic molecules in bulk samples (Fig. 6) can be explained by the fact that these samples still contained a fair amount of undecomposed plant remains, and that part of the humified material may still be closely related to such remains. In addition, it is likely that the dominant presence of mineral particles inhibited the same close contact among organic molecules as in the extracts.

The possibility that the conspicuous shift to more than 800 °C of the exothermic peak of Na-humates in methanol would be due to molecular artifacts formed during high-temperature reactions with methanol was ruled out by the series of experiments shown in Fig. 7. The same B-horizon Na-humate sample that showed the high-temperature shift in methanol was subjected to different treatments to estimate the reversibility of the temperature shift. A further stabilization was observed when the sample that was previously wetted with methanol was either oven-dried at 50 °C for several days, or wetted again with water and oven-dried. Conversely, when the sample was freeze-dried after rewetting with water, an almost complete reversal to the thermal profile of the untreated Na-humate was observed (Fig. 7).

Also these observations are explained by the molecular flexibility of Na-humates. When Na-humates are wetted again with water after removal of residual methanol by oven-drying (50 °C), water favours both a re-hydration of inner humic sites and redistribution of the most hydrophilic molecules within humic aggregates. However, oven drying the sample again causes evaporation of the hydration water with the concomitant separation of hydrophilic from hydrophobic components. The thermal stability of the latter is again enhanced. Conversely, the freeze-drying process prevents the relative rearrangement of hydrophilic and hydrophobic components, since hydration water sublimizes without affecting the distribution of hydrophilic molecules in humic associations. Therefore, the freeze-drying process reduces the molecular rearrangement that leads to an increased hydrophobicity and consequent increased thermal stability of humic materials.

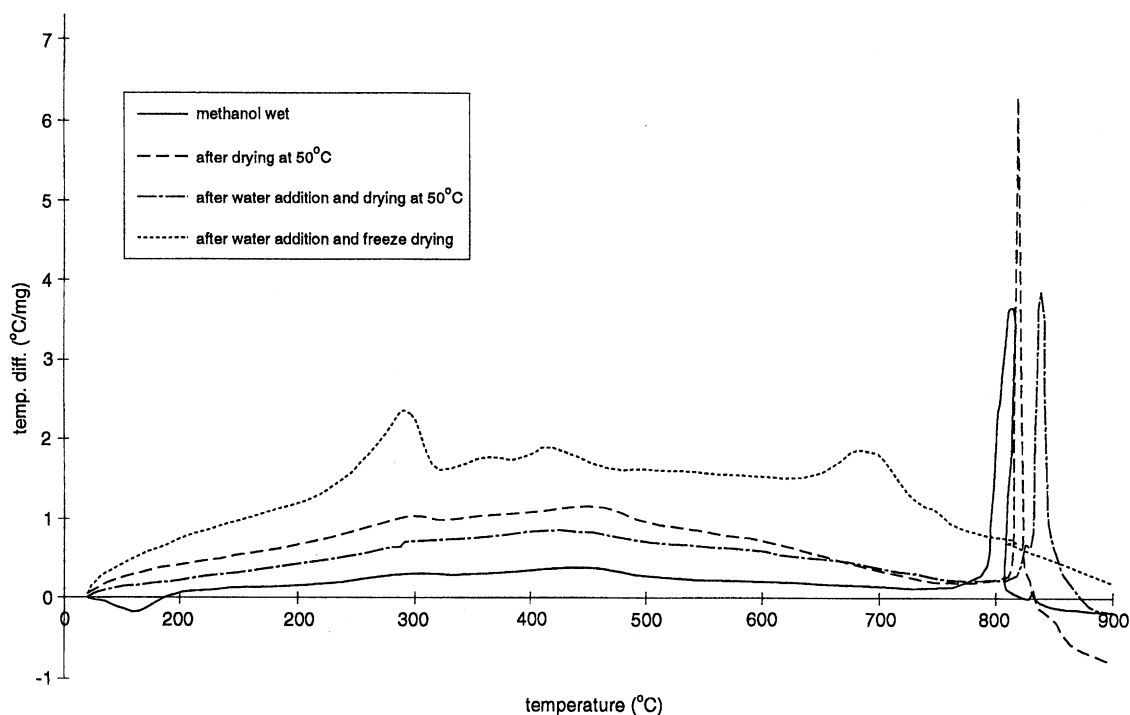


Fig. 7. Effect of various treatments on the stability of rearrangement with methanol of Na-saturated B-horizon extracts.

This behaviour accords with the common practical knowledge that the hydrophobicity of humic substances make them difficult to redissolve in water after having been air-dried, whereas dissolution is facilitated when they are freeze-dried (Swift, 1989).

All the above findings support a model of the self-associating supramolecular nature of humic molecules (Piccolo and Conte, 2000; Conte et al., in press). The ease with which rearrangement occurs in Na-humates suggests that the humic molecules have small molecular dimensions rather than being macromolecular polymers. It is unlikely that the sharp and intense exotherm observed above 800 °C could be due to the coiling down of a large polymer, nor that such a polymer could resume its original thermal profile after freeze-drying. In fact, Ca- and Al-humates, which have been reported to have much larger aggregate size (Yates and von Wandruszka, 2000), did not produce the same high-temperature shifts or the reversible behaviour observed for the Na-humates.

The effect of formic and acetic acid on the thermal behaviour of the different humates can also be explained by the solvophobic properties of these solvents on solid surfaces. Both formic and acetic acid have higher structural organization than methanol because their carboxylic functions form intramolecular hydrogen bonds (Tanford, 1991) and molecules are packed as dimers in the bulk liquid (Israelachvili, 1992), as reflected by their different boiling points (64.9, 100.7, and 117.9 °C, for

methanol, formic acid, and acetic acid, respectively). The molecular rearrangement occurring by wetting humates with methanol would not take place to the same extent in the organic acids, because the latter would be less available to solvate hydration water and hydrophilic groups from humic associations. A number of exothermic peaks at higher temperatures than the control sample were still observed upon wetting with organic acids, but less significant than in methanol treatment (Fig. 3a and b). The effect of acetic acid was still visible in the thermal profiles of the Ca-humates, where a change in relative intensity of the exotherms was noted (Fig. 4a and b). In most cases, a better definition of exotherms was obtained, indicating some rearrangement. In the more rigid Al-humates, additions had hardly any affect at all.

## 5. Conclusions

The present study has shown that the thermal stability of humic substances depends not only on the counter-ion neutralizing the negative charges of the most acidic functional groups, but also on the organic compounds which may come in contact with humus. In the case of sodium-humates, the thermal stability of humic conformations is increased substantially when organic solvents such as methanol or small organic acids enhance the hydrophobic forces holding the humic

molecules together. The intermolecular linking between small humic molecules provided by divalent and trivalent cations such as in Ca- and Al-humates increasingly reduces the effect of organic solvents and the relative enhancement of thermal stability. Polysaccharides do not appear to participate in the thermally stable arrangement.

Although the effect on bulk samples was less pronounced, the consequences of these findings may increase the understanding of organic matter behaviour in natural conditions. The conformational arrangement brought about by organic compounds may be the cause of a higher organic matter stability when low-molecular-weight organic acids are abundant as root exudates, and in environments with Na-saturated organic matter. Indications of the latter can be found in Surapaneni et al. (1998) and Gonzalez-Prieto et al. (1992). The former authors investigated the effect of Na-salinization on the amount of easily-oxidizable organic matter in some soils of Southern Australia, and found that the easily-oxidizable fraction was appreciably reduced with increasing exchangeable Na. The latter authors reported a negative effect of exchangeable Na on nitrogen mineralization in soils of temperate-humid Spain. A decrease in carbon mineralization with increased sodicity in soil-straw mixtures was also observed by Nelson et al. (1997).

Our results suggest that an additional mechanism of humus stabilization in soil may be hypothesized besides the formation of complexes with di- and tri-valent cations. Humus stabilization against microbial degradation may also occur by a progressive, non-specific hydrophobic aggregation induced by low-molecular-weight organic acids exuded by plant roots.

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