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Abstract

Mt Etna (Italy) is the highest, active volcano in Europe. Thanks to the availability of descriptive documents and maps, it is possible to date the formation of several Etna soils.

We evaluated the effect of soil formation age on the composition of the soil organic matter (SOM) and humic acids (HAs) from two Etna andisols of different ages but having the same vegetation (*Catsanea sativa*) and pedogenetic substrate (tephra). The characterization of the SOM and the HAs was carried out by elementary and functional group analysis, UV-visible spectrophotometry, Fourier Transform InfraRed (FT-IR) and [1]H-Nuclear Magnetic Resonance (^{1}H-NMR) spectroscopies, and Pyrolysis - Field Ionization Mass Spectrometry (py-FIMS). Data showed that the age of the soil cannot be considered as influencing the chemical composition of the SOM. However higher volatilisation temperatures, for some compound classes, indicate the presence of more thermally-stable, organic molecules, therefore a more mature organic matter, or an organic component more greatly bound to the mineral component in the older soil.

Finally the HA extracted from old soil contains a lower quantity of aliphatic compounds and a greater quantity of carbohydrate and aromatic molecules than the HA extracted from young soil.

1. INTRODUCTION

The active volcano Mt Etna, situated in the province of Catania, Sicily, is the highest in Europe (about 3350 m). Its present morphology is the result of many consecutive effusive and explosive events whose products overlap each other, often making it difficult to upgrade cartographic details. The vegetation of the volcano is diverse as a consequence of considerable variation in climate. It is also dependent on altitude, the exposure to and the effects produced by volcanic activity and, above all, the influence of anthropogenic pressure in the lower areas.

Thanks to documents and maps describing the various eruptions, stratigraphic [1-2], petrological [3] and radiometric determinations [4] and the geology studies of the territory [5], it is possible to date several of Etna's soils.

Besides the prevalent presence of Entisols at higher altitude, the soils from medium altitude are frequently classified as Andisols [6-8]. According to Duchaufour [9] the Andisols (so-named by USDA Soil Taxonomy) typically form on volcanic ash and are essentially constituted of allophane-organic matter complexes. These soils also form on erupted hard rock containing fine crystalline minerals and amorphous materials that can be rapidly weathered. On the other hand, slower weathering will cause the formation of a shallow soil with a lower content of organic matter, thus showing the typical characteristics described by Duchaufour [9].

Because of large specific surface area in the range of 700-1100 m^2 g^{-1} [10], the allophane can establish high proportions of partially evolved organic matter and protect it from microbial biodegradation which causes an accumulation of organic matter in the soil profile.

The extensive formation of organo-mineral complexes depends on climatic conditions such as almost-constant humidity. Otherwise the irreversible evolution of the amorphous mineral and organic substance causes the formation of another kind of soil, andic soil or andic Inceptisol (e.g.,

Andic Haploxerept) [9]. Moreover, parent material also influences the formation of organic-mineral complexes. On the contrary, the product resulting from the decomposition of the vegetation litter plays a minor role [9].

The formation age of the soil influences the nature and the quantity of mineral alteration products [11].

Several researchers [12-17] have extracted humic acids (HAs) from Andisols and investigated them by elemental analysis (C, H, N), functional group titrations, and UV-visible, Fourier Transform InfraRed (FT-IR), ^{13}C-Nuclear Magnetic Resonance (^{13}C-NMR) and [1]H-Nuclear Magnetic Resonance (^{1}H-NMR) spectroscopies. In these studies the authors concluded that the HAs from Andisols were characterised by high aromaticity. This characteristic of HAs extracted from Andisols is also revealed by other methods: pyrolysis-Field Ionization / Mass Spectrometry (py-FIMS; [18]), optical density of visible spectra [19] and X-ray diffractometry [20].

Kuwatsuka et al. [21] and Yonebayashi and Hattori [22-23] (1988, 1989) extracted HAs from Andisols collected in Japan and concluded that they were characterised by a high degree of humification, as defined by Kumada et al. [24] from optical densities ratios at 400 and 600 nm.

Leinweber et al. [25] studied the molecular composition of soil organic matter (SOM) in Pleistocene moraines of different ages (9,000, 16,000, 20,000 and >20,000 years) from the Bolivian Andes by elemental analyses and py-FIMS. The authors concluded that: 1) relative enrichments of resistant lipids, sterols, fatty acids and nitrogen-containing compounds and 2) an increase in thermal stability, indicating the strengthening of chemical bonds as a significant process during SOM aging, occur with increasing soil age.

However in other works Schulten and Leinweber [26-27] observed that some SOM compounds bounded to inorganic soil matrix, above all clay minerals and iron oxides, exercised a great influence on the thermal stability.

Watanabe and Takada [28] examined the relationships between soil age (eleven soil layers with ages comprised between 3000 and 30000 years) and the structural stability of humic acids in

volcanic ash soil profiles taken near Mt Fuji (Japan), by the composition of C functional groups, solid state ^{13}C-NMR spectroscopy, and the degree of humification (as defined by [24]). The authors observed that with increasing soil age the proportion of aromatic C in total C of the humic acids, increased, whereas the proportions of alkyl and O-alkyl C decreased. These observations suggested higher stability in the aromatic than the aliphatic components. Lastly, the composition of the C functional groups did not vary appreciably.

We evaluated the effect of soil formation age on the composition of the SOM and HAs from two Etna andisols of different ages but having the same vegetation and pedogenetic substrate. The characterization of the SOM and the HAs was carried out by elementary and functional groups analysis, UV-visible spectrophotometry, FT-IR and ^{1}H-NMR spectroscopies, and py-FIMS. The py-FIMS can be regarded as a very successful tool because of the increasing proportions of volatilized and identified C found by this means [29].

This, and information about bond strength, obtained from thermograms, make py-FIMS valuable for investigations of soil chronosequences complementary to ^{13}C–NMR spectroscopy [25].

2. METHODS

2.1. Soils

Two Andisols, collected from the east side of Mount Etna, were studied.

The soils indicated as OTS and RTS formed respectively on very old tephra (beginning of pedogenesis about 8700 years ago) and on recent tephra (beginning of pedogenesis less than 3600 years ago), and are both covered with chestnut (*Castanea sativa*).

Samples were taken on the same day, in winter, from horizons: A1, A2 and Bw of OTS; A1, A2 and Bw1 of RTS. Only Horizon A was analysed for its HA composition in the present study. Some details related to the sites and soils are shown in Table 1.

Table 1

2.2. Extraction and characterization of HAs

HA extraction was carried out on soil using 0.5 M NaOH and 0.1 M $Na_4P_2O_7$ (NaOH + NaPP) solution, according to the IHSS procedure [30]. The suspension was de-aerated with N_2, shaken for 20 h and centrifuged at 1500g for 30 min; the supernatant was collected and the solid residue was extracted again as above. The procedure was repeated three times. The pooled supernatants were filtered, acidified with 6 M HCl to pH 1 and left overnight. The resulting brown precipitate was separated from the liquid phase by centrifugation (1500g, 30 min), resuspended in NaOH + NaPP, de-aerated with N_2 and shaken overnight. After centrifugation (1500g, 30 min), the liquid phase was acidified with 6 M HCl to pH 1, left to stand overnight and recentrifuged. The precipitate was washed in distilled water, dialysed against distilled water (3500 dalton MW cut-off) and freeze dried.

2.3. Soil characterization

The soils were air-dried and sieved at < 2 mm. The pH (H_2O) and the texture of soil were measured according to the Italian Soil Science Society methods [31], while the elementary analyses were performed using a CHN-analyzer 600 (LECO Corp. St. Joseph, MI, USA).

For py-FIMS about 5 mg of soil sample were transferred to a quartz mini-oven (AMD Intectra GmbH, 27243 Harpstedt, Germany) connected to the ion source of a MAT 731 mass spectrometer (Finningan, 28127 Bremen, Germany). The instrumental set-up is reported in detail by Schulten [32]. The samples were weighed before and after analysis to determine the pyrolysis residue and volatile matter. The heating/cooling system was electronically programmed and the potential of the ion source and the field ionisation emitter used were 8000 m^2 kg s^{-3} A^{-1}. The cathode in place served as a counter cathode and was set to a potential of – 6000 m^2 kg s^{-3} A^{-1}. The distance between the electrode and the emitter was 2 mm, so as to have an applied potential difference of 14000 m^2 kg s^{-3} A^{-1} and high electric field energy, essential for soft ionisation. The samples were heated in a high vacuum (1.3 x 10^{-4} m^{-1} kg s^{-2}) from 100 to 700 °C with a heating rate near to 0.17 °C s^{-1}. About

60 scans of the pyrolysis product, present in the gaseous phase, were carried out over an interval of 15 – 1000 mass units for each sample. A minimum of three replicates were run for each sample. The total ionic intensity (TII) of each single spectrum was related to 1 mg of sample, then the TII per mg sample was averaged out for the replicates, and the resulting mean TII was plotted against the pyrolysis temperature, thereby providing the py-FIMS thermogram of TII. The TII thermograms describe the evolution of thermal products and are characteristics of the composition and bonds of the constituents released by the humic macromolecule [18]. Detailed descriptions of the py-FIMS methodology, volatilized matter and total ion intensities have been given by Schulten [32-33], and Sorge et al., [34].

2.4. HA characterization

The total acidity and the content in carboxyl groups were determined by titration according to the procedure proposed by Swift (1996). The content of C, H and N was determined using a C, H, N Analyzer mod.600 (LECO Corp., St. Joseph, MI), and the E_4/E_6 ratio was determined in accordance with Chen and Pawluk [38] using a Cary 1E UV-visible spectrophometer. The FT-IR spectra of pellets (0.5 mg of HAs and 200 mg of KBr) were obtained with a Perkin-Elmer FT-IR 1600.

The ^{1}H-NMR spectra were recorded using the Presat sequence for selective saturation of the HDO resonance [35]. The main NMR parameters were: 45° pulse width, pre-irradiation 10 s, acquisition time 5.46 s and 64 scans for a total acquisition time of about 16 min. Py-FIMS spectra of HAs were obtained on 100 μg of sample as described above.

3. RESULTS AND DISCUSSION

3.1. Soils analyses

Table 2 lists the basic characteristics of the soils. They have a sub-acidic reaction and sandy texture similar to several andisols reported by Duchaufour [9]. OTS showed a higher carbon

content, and similar N content compared with RTS resulting in a slightly higher C/N ratio. The C content of the soils was lower than that reported by Duchaufour [9] for andisols covered by forest (about 8 – 12 %).

Table 2

3.1.1. Pyrolysis-Field Ionization Mass Spectra of soil organic matter

Fig. 1 shows the thermograms of TII (upper right) and the summed and averaged Py-FI mass spectra (lower left). Table 3 shows the TII (in 10^6 counts mg^{-1}) and the relative intensity (% TII) of ten compound classes.

Fig. 1

The attribution of the signals (m/z) is based on the works of Schulten and Simmleit [36], Hempfling et al. [37] and Schulten and Schnitzer [18].

The thermograms of total ion intensities (TII) and the summed and averaged py-FI Mass Spectra show differences in the molecular composition of SOM. In the TII-curves (upper right in Fig.1) two peaks show that the volatilized organic molecules were bound with different stabilities. In RTS the lower-temperature pyrolysis peak appears at about 350°C whereas in OTS it appears at 400°C. This indicates, in accordance with the findings of Leinweber et al. [25], that with aging some portions of the volatilized organic matter of the soil were more strongly bound and that this change occurred mostly with substances which are thermally relatively labile.

The py-FIMS spectra of the two samples are similar. Apart from the non-specific signals (with m/z signals < 58) the signals referring to carbohydrates (m/z 60, 82, 96, and 110) and peptides (m/z 79 and 84) are more intense in both the samples at lower m/z values (Fig. 1). As the m/z values increase the 252 m/z signal, attributed to the lipids, is more intense in both the samples, while the 270 m/z and 284 m/z signals, attributed to n-fatty acids, are more intense in RTS (Fig.1).

For a more detailed insight into SOM composition, the relative abundances of important compound classes were calculated. In comparison to similar data evaluations in former studies [26,34], here we

considered ten compound classes, including carbohydrates, phenols+lignin monomers, lignin dimers, lipids (lipids, alkanes, fatty acid, *n*-alkil esters), alkylaromatics, N-containing compounds (heterocyclic nitrogen compounds), sterols, peptides, suberin and *n*-fatty acids ($16 < n < 34$) (Table 3). The sum of assigned compounds were similar in both samples (42.4% and 41.5% in OTS and RTS respectively). Moreover, there are no significative differences in either soil sample between the ten compound classes considered. So the soil age cannot be considered as influencing the chemical composition of the SOM.

Table 3

The thermograms for the volatilization of ten important compound classes of SOM are shown in Fig.2. The scales at the ordinates show, that in OTS the ion intensities of phenols+lignin monomers, lignin dimers, lipids alkylaromatics, N-containing compound, sterols and peptides, were slightly larger than in RTS.

Fig. 2

The curve shapes for lignin dimers, lipids, sterols and suberins were bimodal and it can be seen that the first and second volatilisation peaks occured at higher temperatures in OTS compared to RTS. These higher volatilisation temperatures indicate the presence of more thermally stable organic molecules, therefore a more mature organic matter or an organic component more greatly bound to the mineral component of the older soil. This last hypothesis seems quite probable considering the quantity of organo-mineral compounds in the andisols [9].

Finally, from the thermogram referring to the n-fatty acids it can be observed that these volatilise in a very narrow temperature range (between 280 and 420 °C) in both samples. This result shows a considerable homogeneity of composition in this class of compounds.

3.2. Humic acids

3.2.1. Basic analyses

The yield values are similar for the two HAs (0.19 and 0.18 HA Carbon/Soil Carbon) and are in agreement with Chen and Pawluck [38] for other HAs extracted from soils of different origins, which have yields in the range 0.16 to 0.30 (HA Carbon/Soil Carbon). The C, H and N contents are also similar for the two HAs (Table 4). Consequently, the H/C ratio and the C/N ratio for the two HAs are similar in RTS and OTS.

Table 4

The C contents are lower than those reported for HAs from andisols (> 57 % C; [13,17,18,22]), while the H contents are slightly greater than the values generally reported in the above literature (> 4 %). The carboxyl and the phenol-OH group contents in the HAs are slightly greater for OTS than RTS (Table 4). In general the COOH group content is lower compared to the literature data for HAs extracted from andisols (between 4.6 and 6.6 meq/g; [13,17,18,22]) whereas the phenol-OH groups content is higher than that reported by Yonebayashi and Hattori [22] and Yonebayashi [39], who found values for this functional group between 0.7 and 1.6 meq/g.

The differences to be seen between our HAs and those studied by other authors as regards the content of acid groups can be attributed: a) to the diverse origins of the humic acids and their different molecular dimensions [40]; b) to diverse climatic zones in which the HAs formed [41]. In fact, Schnitzer [41] found COOH values between 3.9 and 4.5 meq/g in Cool and Temperate climatic zones, values between 4.2 and 5.2 meq/g in Subtropical conditions, and values around 3.2 meq/g in Artic climatic zones.

Table 4 shows that the E_4/E_6 ratio is slightly greater for the RTS-HA compared to OTS-HA. In agreement with Stevenson [42] and Nègre et al. [17] who obtained an inverse relationship between the E_4/E_6 ratio values and the degree of aromaticity of the HAs, these data would suggest a greater aromaticity in OTS-HA, although this is not confirmed by a lower value of the H/C ratio. In fact,

according to Chen and Pawluck [38] and Fujitake and Kawagashi [16] the H/C ratio is inversely proportional to the degree of aromaticity.

The FT-IR spectra (Fig. 3) of HAs from both OTS and RTS show characteristic bands at 2912 cm^{-1} and 2848 cm^{-1} (attributed to the stretch of CH$_2$-groups), 1713 cm^{-1} (attributed to carboxyl groups), 1655 cm^{-1} (attributed to aromatic C=C and primary amides), at 1515 cm^{-1} (attributed to secondary amides) and at 1070 cm-1 (attributed to C-O stretching of polysaccharide or to Si-O of silicate impurities). The assignment of the bands was based on the work of Stevenson and Goh [43], Lin-Vien et al. [44] and Swift [30]. However, it can be seen that the peaks attributed to the stretch of CH$_2$-groups are higher in RTS-HA while the signal referring to polysaccharides is higher in OTS-HA.

Fig. 3

3.2.2. Pyrolysis-Field Ionization Mass Spectra

The TII thermograms (upper right in Fig. 4) show differences in the thermal behaviour between OTS-HA and RTS-HA.

The curve for OTS-HA has a Gaussian-like form with a peak at 460°C, while RTS-HA show a bimodal form (the first volatilisation peaks occurred at about 400°C and the second occurred at about 480°C). The presence of the first very intense volatilisation peak at a relatively low temperature indicates the presence of an organic component with a low level of humification and low aromatic character in the RTS-HA [18].

Fig. 4

The py-FIMS mass spectra of HAs from OLS and RLS are similar in the predominance of carbohydrates (e.g. *m/z* 84, 110, 162), and phenols + lignin monomers (e.g. *m/z* 124, 196, 212) in the lower mass range, and of lipids (e.g. *m/z* 244, 258) and alkyl aromatics (e.g. *m/z* 244) in the higher mass range. However, there are clear differences in the signal patterns between the OTS-HA

and the RTS-HA. The signals in the mass range $m/z > 250$ (attributed to lipids and n-fatty acids) are much more intense in the RTS-HA than in the OTS-HA (Fig. 4).

Table 5

Data in Table 5 show that the RTS-HA has a greater TII and greater proportions of lipids, sterols, suberins and free fatty acids, in agreement with the observations made regarding the TII curves (Fig. 4). Moreover the RTS-HA is lower in heterocyclic nitrogen-containing compounds, phenols+lignin monomers, lignin dimers and alkyl aromatics than the OTS-HA. The smaller lipid content in the OTS-HA compared to the RTS-HA is in agreement with the findings of Leinweber et al. [25], who found relative lipid enrichments in more recent soils in a sample set from the Bolivian Andes.

The thermograms for the volatilisation of compound classes in Fig. 5 show similar forms and widths of curves only for carbohydrates, for phenols+lignin monomers and for peptides. This indicates that these compound classes were homogeneous in the two HAs.

Fig. 5

Lignin dimers have a volatilisation maximum at a surprisingly higher pyrolysis temperature (about 510°C) in the RTS-HA than in the OTS-HA (about 470°C), this class of compound in the HA extracted from the younger soil thus proving more thermally stable. The thermograms for the volatilisation of alkyl aromatics, lipids and N-containing compounds have a Gaussian-like form for the OTS-HA and a bimodal form in the RTS-HA. The thermograms of lipids, sterols and fatty acids in the RTS-HA cover wider temperature ranges than those of these compound classes in the OTS-HA. This indicates that there is a tendency towards thermally more homogeneous structures with soil development.

Fig. 6

3.2.3. Liquid state ^{1}H-NMR

Fig. 6 shows the ^{1}H NMR spectra of the HAs. This technique proved easier to use with samples having higher ash content or paramagnetic ions than did ^{13}C NMR. Nevertheless, ^{1}H NMR tends to underestimate the aromaticity, above all when the aromatic molecules are substituted by atoms other than hydrogen [42].

No differences can be seen in the form of the two spectra (Fig. 6). The most evident peaks (0.8, 0.9, 1.3, 1.5, 1.9, 2.0, 2.1 and 2.2 ppm) are present in the region of the H forms linked to aliphatic groups in both samples. Nevertheless the peaks at 1.3 and 1.5 ppm, attributed to methylene of methylene chains or methylene of alicyclic compounds [45-46] are higher in the RTS-HA, while the peaks at 1.9 and 2.0 ppm, attributed to protons of methyl and methylene groups α to aromatic rings [45-46] are higher in the OTS-HA.

The distribution of the different forms of hydrogen, reported in Table 6, was calculated by integration into three regions of the spectrum (0.5–3 ppm, 3–4.2 ppm and 6–8 ppm) and expressed as a % of the total area of each spectrum. The allocation of the three regions was based on the work of Francioso et al. [47]; in particular, the region from 0.5 to 3 ppm corresponds to the H forms linked to aliphatic groups, the region from to 4.2 ppm is assigned to the H forms linked to carbohydrate structures and finally the region between 6 and 8 ppm is allocated to the H forms linked to aromatic molecules.

Table 6

From Table 6, which relates to the distribution of the ^{1}H forms, it can be seen that the OTS-HA, contains a lower quantity of aliphatic H and a greater quantity of carbohydrate and aromatic H than the RTS-HA, in agreement with the FT-IR spectra and the E_4/E_6 ratio. A great presence of carbohydrates was also found in the OTS-HA with py-FIMS analyses (Table 5).

Allard [48] who studied the chemical nature of HAs extracted from different soils, also observed that a greater carbohydrate content was accompanied by higher degree of aromaticity.

An increase in the proportion of aromatic C in humic acids as the age of the soil increases was also observed by Watanabe and Takada [28] who suggested that the aromatic components had a greater

structural stability than the aliphatic components. The carbohydrate material also seems to be very stable in that it is firmly bound to the humic colloids in the form of polysaccharides [42].

Therefore, more carbohydrates and aromatic molecules lead to higher number of organic-mineral complexes, of which many are to be found in the andisols.

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Figure captions

Figure 1. Thermograms of total ion intensity (in the rectangles on the right side) and Py-FIMS mass spectra of soils.

Figure 2. Thermograms for the volatilisation of different classes of compounds in soil (10^6 counts mg^{-1}).

**Figure 3.** Fourier Transform InfraRed spectra of humic acids extracted from OTS and RTS.

Figure 4. Thermograms of total ion intensity (in the rectangles on the right side) and Py-FIMS mass spectra of humic acids extracted from OTS and RTS.

Figure 5. Thermograms for the volatilisation of different classes of compounds in humic acid (10^6 counts mg^{-1}).
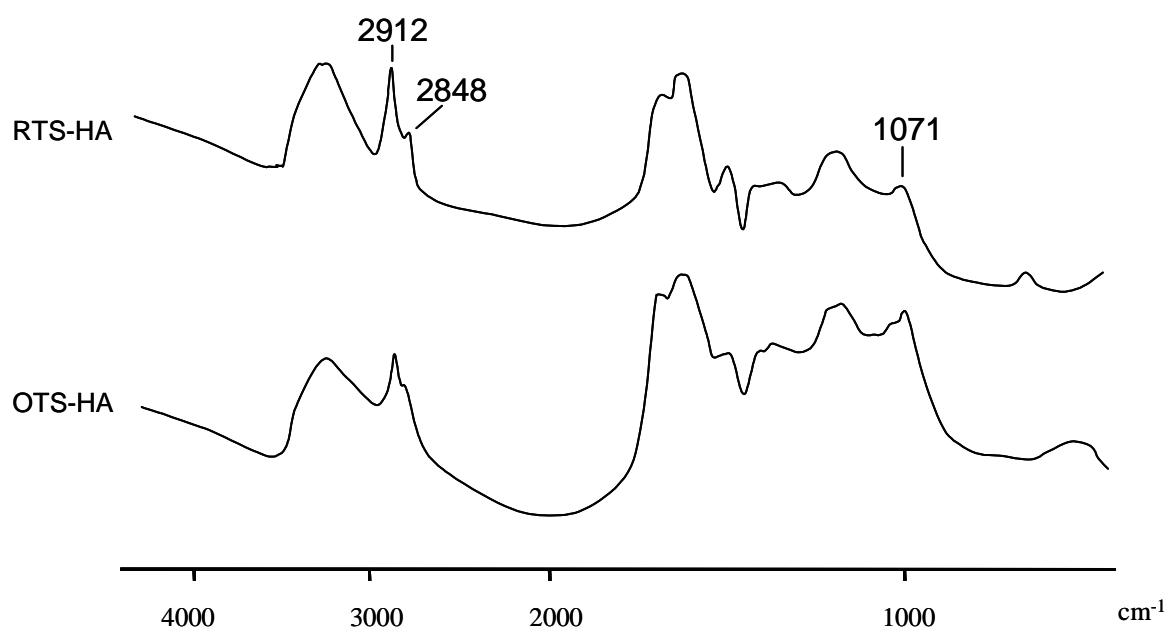
Figure 6. ^{1}H-NMR spectra of humic acids extracted from OTS and RTS.
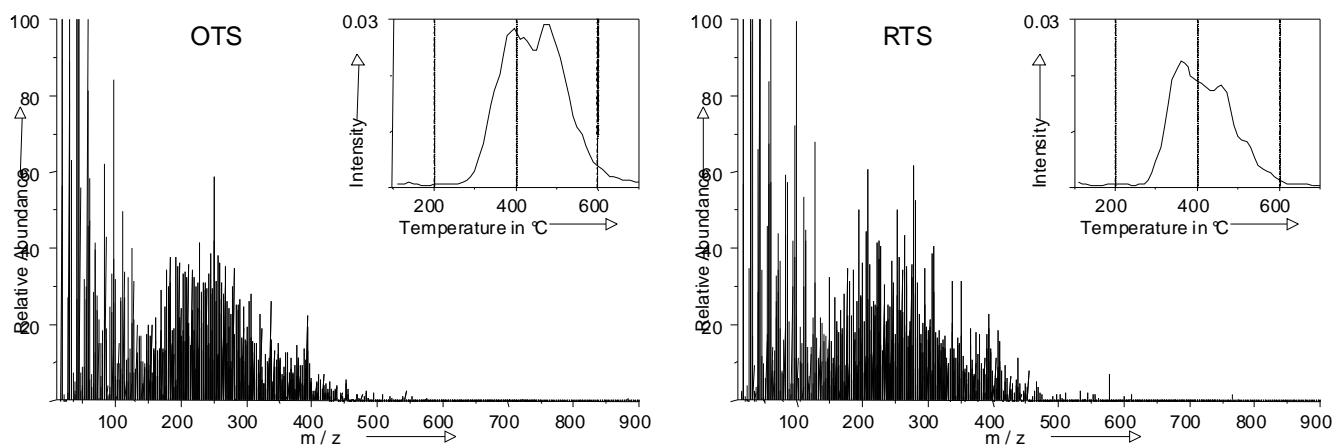
A. Baglieri, M. Gennari, A. Ioppolo, P. Leinweber and M. Nègre. **Figure 1**.
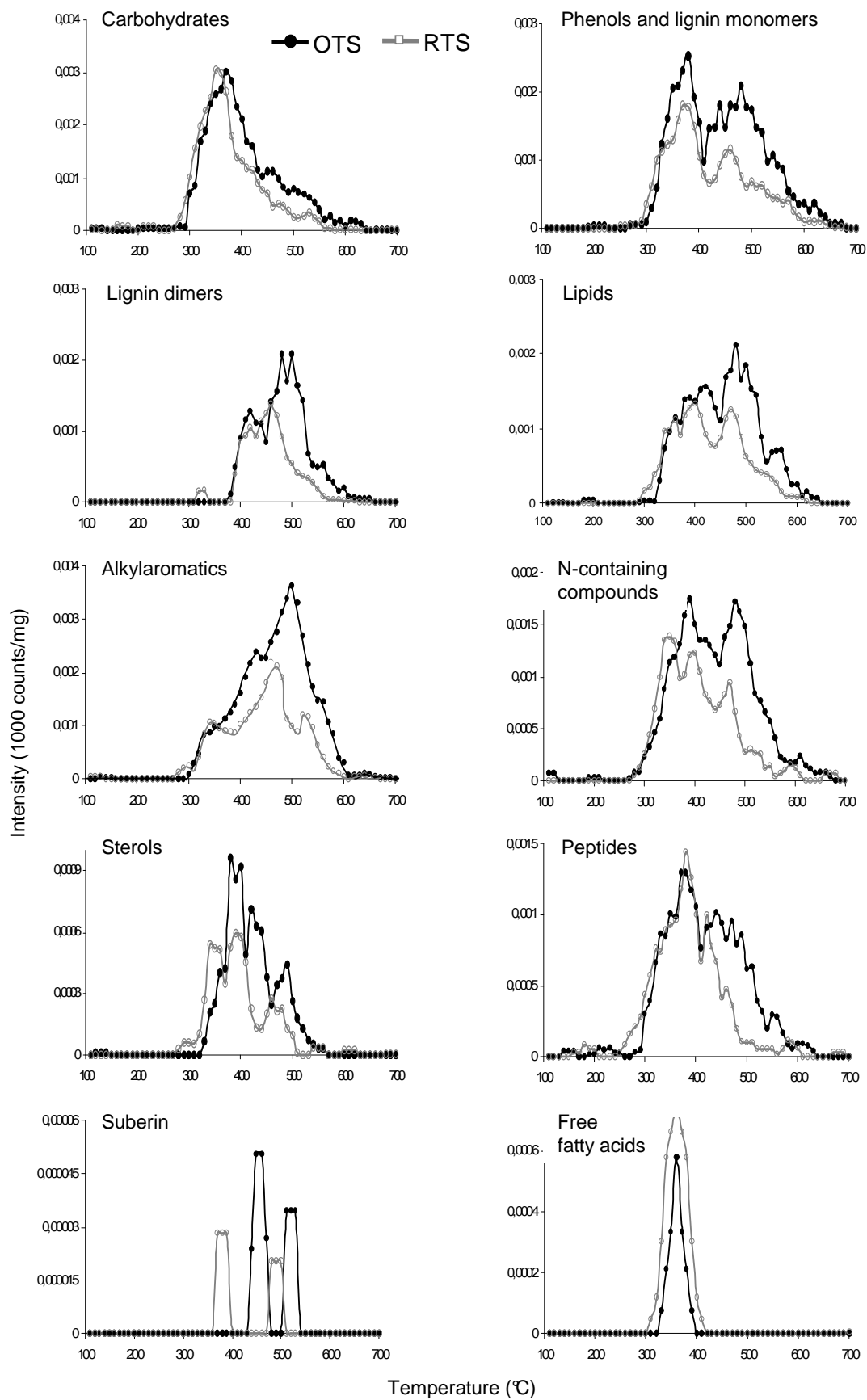
A. Baglieri, M. Gennari, A. Ioppolo, P. Leinweber and M. Nègre. **Figure 2**.

A. Baglieri, M. Gennari, A. Ioppolo, P. Leinweber and M. Nègre. **Figure 3**.
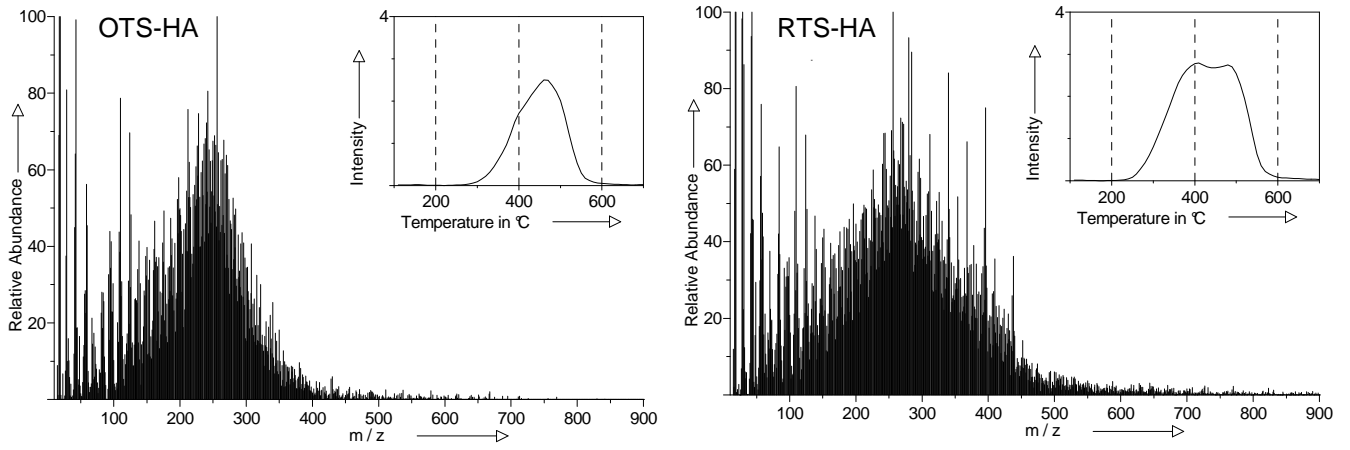
A. Baglieri, M. Gennari, A. Ioppolo, P. Leinweber and M. Nègre. **Figure 4**.
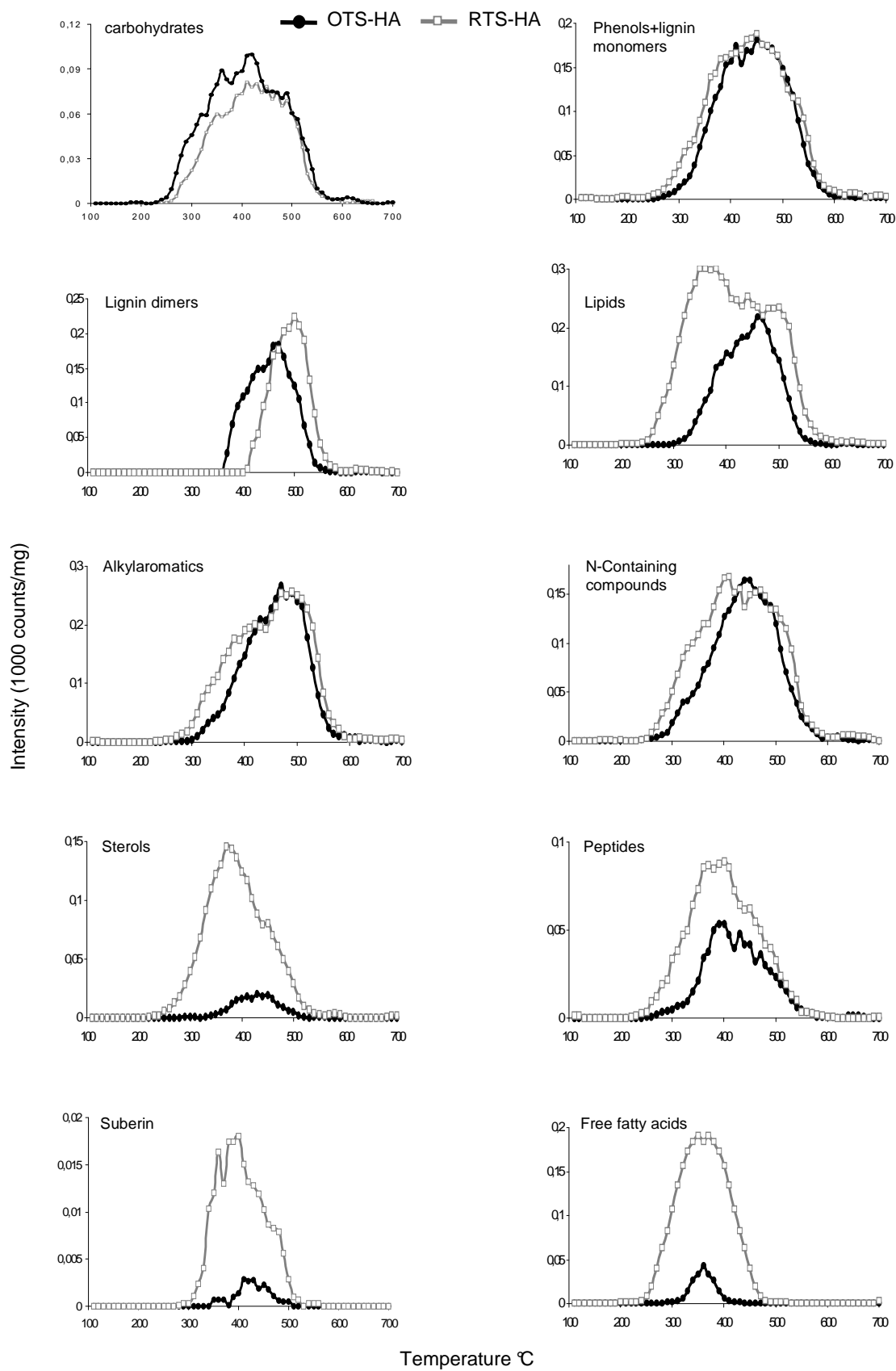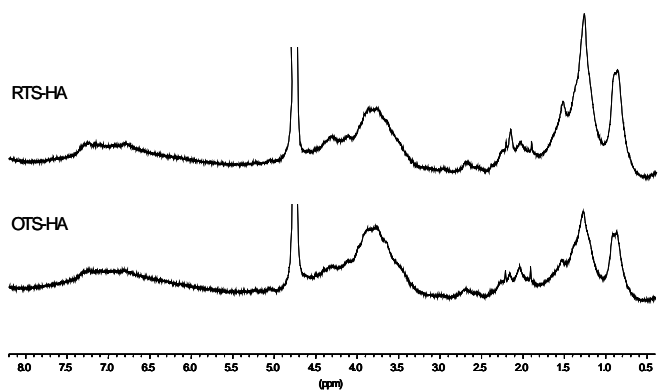
A. Baglieri, M. Gennari, A. Ioppolo, P. Leinweber and M. Nègre. **Figure 5**.

A. Baglieri, M. Gennari, A. Ioppolo, P. Leinweber and M. Nègre. **Figure 6**.

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2 **Table 1**. Soils from Mt Etna

	Site and volcano sector	Altitude (m)	Pedon profile	Classification (Soil Taxonomy)	Dominant vegetation
OTS	Razzara W-NW of Zafferana: sector S-E	1130	Oi-Oe/C-A1-A2-Bw-C-2A-2B-2C-3A	Typic Udivitrand	*Castanea sativa*
RTS	Mt Monaco W-NW of Zafferana: sector S-E	1400	Oi-C-Oe/C-A1-A2-Bw1-Bw2	Typic Udivitrand	*Castanea sativa*

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13 **Table 2**. Characteristics of soil samples

Soil	pH	Coarse sand[z] (%)	Fine sand[y] (%)	Coarse silt[x] (%)	Fine silt[w] (%)	Clay[v] (%)	Elemental composition		
							N (%)	C (%)	H (%)
OTS	6,5	68,3	24,3	0,9	4,7	1,8	0,1	1,7	0,5
RTS	6,4	74,7	20,1	1,5	3,5	0,2	0,1	1,1	0,3

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15   [z] 2.0÷0.20 mm ;
16   [y] 0.20÷0.05 mm ;
17   [x] 0.05÷0.02 mm ;
18   [w] 0.02÷0.002 mm ;
19   [v] < 0.002 mm ;
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Table 3. Total ion intensities, volatile matter and relative ion intensities of ten important compound classes of soils.

Soil	TII[z] 10^6 counts (mg^{-1})	$(mg^{-1}C_{org}^{-1})$	VM % (w/w)	CHYD	PHLM	LDIM	LIPID	ALKY	NCOMP	STERO	PEPTI	SUBER	FATTY	Sum
OTS	0.60	35.1	11.9	6.6	7.2	3.7	5.5	8.7	5.2	1.5	3.6	0.0*	0.3	42.4
RTS	0.41	37.63	11.6	7.8	6.4	3.2	5.2	7.3	4.9	1.6	4.0	0.0*	1.0	41.5

[z]TII = total ion intensity; VM = volatile matter; CHYD = carbohydrates; PHLM = phenols+lignin monomers; LDIM = lignin dimers; LIPID = lipides; ALKY = alkylaromatics; NCOMP = heterocyclic nitrogen containing compounds; STERO = sterols; PEPTI = peptides; Suber = suberin; FATTY = free fatty acids.
[y]The numbers in **bold type** indicate statistical significance (t-test one sided, significant P < 0.05) in the columns.
* < 0.1 %

Table 4. Characteristics of extracted humic acids

HA	HAC[z]/ soil C	Ash (%)	N (%)	C (%)	H (%)	O[y] (%)	H/C	C/N	Carboxyl	OH-Phenol	Total acidity	E_4/E_6
			Elemental composition				Molar ratio		Acidic groups (meq g^{-1})			
OTS	0.19	0.6	7.6	51.7	4.2	30.0	1.0	7.8	2.7	3.7	6.4	5.2
RTS	0.18	0.6	3.7	52.6	4.6	34.5	1.0	16.6	2.3	3.1	5.4	5.8

[z]Humic acid Carbon;
[y]Oxygen determined by difference, correcting the value for the humidity and ash ;

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9 **Table 5**. Total ion intensities, volatile matter and relative ion intensities of ten important

10   compound classes of humic acids.

HA	TII[z]		VM	CHYD	PHLM	LDIM	LIPID	ALKY	NCOMP	STERO	PEPTI	SUBER	FATTY	Sum
	10^6 counts (mg^{-1})	(mg^{-1}C$_{org}^{-1}$)	% (w/w)											
OTS	**35.9**	**69.4**	54.4	4.2	**8.7**	**5.8**	**7.9**	**10.4**	**7.1**	**0.6**	2.1	**0.1**	**0.6**	47.3
RTS	**59.2**	**112.5**	54.5	3.2	**6.2**	**3.4**	**10.7**	**7.9**	**5.6**	**3.4**	2.5	**0.4**	**4.3**	47.5

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12 [z]TII = total ion intensity; VM = volatile matter; CHYD = carbohydrates; PHLM = phenols+lignin monomers;
13   LDIM = lignin dimers; LIPID = lipids; ALKY = alkylaromatics; NCOMP = heterocyclic nitrogen
14   containing compounds; STERO = sterols; PEPTI = peptides; Suber = suberin; FATTY = free fatty acids.
15 [y]The numbers in **bold type** indicate statistical significance (t-test one sided, significant $P < 0.05$) in the columns and
16 between the sample groups (LO vs LR and TO vs TR)

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2 **Table 6**. Relative intensity distribution in the ^{1}H-NMR spectra of the humic acids

	^{1}H-NMR distribution (%)		
	Aliphatic H 0.5-3.0 (ppm)	Carbohydrate H 3.0-4.2 (ppm)	Aromatic H 6.0-8.0 (ppm)
OTS	32.0	57.0	11.0
RTS	66.4	28.5	5.1

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