Report for C. 2 Action: initial situation

The data are related to initial field soil samples from project sites of Piemonte (Tetto Frati and Grugliasco) and Campania, (Castel Volturno and Prima Luce).

completed analyses

- soil aggregate stability
- SOC and total N of bulk soils and soil aggregates;
- SOM characterization by off line thermochemolysis GasChromatografy-MassSpectrometry: initial situation (for Tetto, Frati, Grugliasco and Castel Volturno);

analyses in progress

- SOM characterization by THM GC MS Prima Luce
- charaterization of soil humic substances

Analytical methodologies

- Soil aggregate stability

A modified procedure of the classical method described by Kemper and Rosenau (1986) was used to separate the water-stable aggregates. Forty grams of the <2.00 mm, air-dried soil samples were put in the topmost of a nest of three sieves of 1.00, 0.50 and 0.25mm mesh size and pre-soaked in distilled water for 30 min. Thereafter the nest of sieves and its contents were oscillated vertically in water 20 times using a 4 cm amplitude at the rate of one oscillation per second. Care was taken to ensure that the soil particles on the topmost sieve were always below the water surface during each oscillation. After wet-sieving, the resistant soil materials on each sieve and the unstable (<0.25 mm) aggregates were quantitatively transferred into beakers, dried in the oven at 50◦C for 48 h, weighed and stored. The percentage ratio of the aggregates in each sieve represents the water-stable aggregates of size classes: 2.00–1.00, 1.00–0.50, 0.50–0.25 and <0.25 mm. Mean-weight diameter in water (MWDw) of water-stable aggregates was calculated as follow

$$MWDw = \sum_{i=1}^{n} Xi\ Wi$$

where Xi is the mean diameter of the *ith* sieve size and Wi the proportion of the total aggregates in the *ith* fraction.

- Extraction of soil humic substances

For the extraction of soil humic substances (HS) a slight modified basic IHSS procedure was adopted on the 2 mm sieved soil samples. The humic materials were isolated by shaking soil sample (100 g) overnight with 1000 ml of 0.1 M NaOHsolution under N2 atmosphere. Differently from the basic procedure, no sodium pyrosphate (Na4P2O7) solution was used, in order to avoid the artificial addition of P material in HS extracts which would affect the subsequent determinations.

After having centrifuged away the supernatant, the solid residue was washed with distilled water until pH 7. The supernatants and washing solution were combined, filtered on a quartz filter (Whatman GF/C), and acidified to pH 5 with concentrated HCl. The isolated HS were purified by a 48 h shaking with 0.1 M HCl/0.3 M HF solution (1:50 w/v). The final residue was dialyzed against deionized water, and freeze dried.

-Off-line THM-GC-MS

Pyrolysis in the presence of tetramethyl ammonium hydroxide (TMAH) is commonly used to study the detailed molecular composition of either natural and synthetic biopolymers. It involves the cleavage of covalent bonds combined with the solvolysis and methylation of ester and ether groups, in complex mixture of organic macromolecules and biopolymers, thereby enhancing the thermal stability of acidic, alcoholic, and phenolic groups and allowing a suitable chromatographic detection of pyrolytic products.

For off line-THM-GC-MS about 2 g of soil samples were placed in a quartz boat with 2 mL of TMAH (25% in methanol w/v) solution. After drying under a stream of nitrogen, the mixture was introduced into a Pyrex tubular reactor (50 cm × 3.5 cm i.d.) and heated at 400 °C for 30 min in a circular oven (Barnstead Thermolyne 21100 Furnace, Barnstead International, Dubuque, IA, USA). The gaseous products from thermochemolysis were flowed into two chloroform (50 mL) traps in series, kept in ice/salt baths. The chloroform solutions were combined and rotoevaporated to dryness. The residue was dissolved in 1 mL of chloroform and transferred in a glass vial for GC-MS analysis. The GC-MS analyses were conducted with a Perkin Elmer Autosystem XL by using a RTX-5MS WCOT capillary column (Restek, 30 m × 0.25 mm; film thickness, 0.25 mm) that was coupled, through a heated transfer line (250 °C), to a PE Turbomass-Gold quadrupole mass spectrometer. The chromatographic separation was achieved with the following temperature program: 60 °C (1 min. isothermal), rate 7 °C min−1 to 320 °C (10 min. isothermal). Helium was used as carrier gas at 1.90 mL min−1, the injector temperature was at 250 °C, and the split-injection mode had a 30 mL min−1 of split flow. Mass spectra were obtained in EI mode (70 eV), scanning in the range 45–650 m/z, with a cycle time of 0.2 s. Compound identification was based on comparison of mass spectra with the NIST-library database, published spectra, and real standards.

For quantitative analysis, due to the large variety of detected compounds with different chromatographic responses, external calibration curves were built by mixing methyl esters and/or methyl ethers of the following molecular standards: tridecanoic acid, octadecanol, 16-hydroxyhexadecanoic acid, docosandioic acid, *â*-sitosterol, and cinnamic acid. Increasing amounts of standard mixtures were placed in a quartz boat and moistened with 0.5 mL of TMAH (25% in methanol) solution. The same thermochemolysis conditions as for compost samples were applied for the standards. The percentage recovery of standards ranged from 82 to 91% of initial amount.

Results

- Soil aggregate stability

The differences in aggregate distribution (Fig. 1) and in aggregate stability (larger MWD) between the soil samples from project site of Castel Volturno, as compared with those from Piemonte, have to be mainly referred to the effects of textural composition. In fact the larger clay content in soil samples from Castel Volturno act as stabilizing agent, while the light textured soils from Piemonte show a lower aggregate stability, with the larger amount of soil fraction accumulating in the finest soil aggregates sizes (< 0.50 mm). The silty soil from Prima Luce is characterized by an intermediate behaviour (Fig 1).

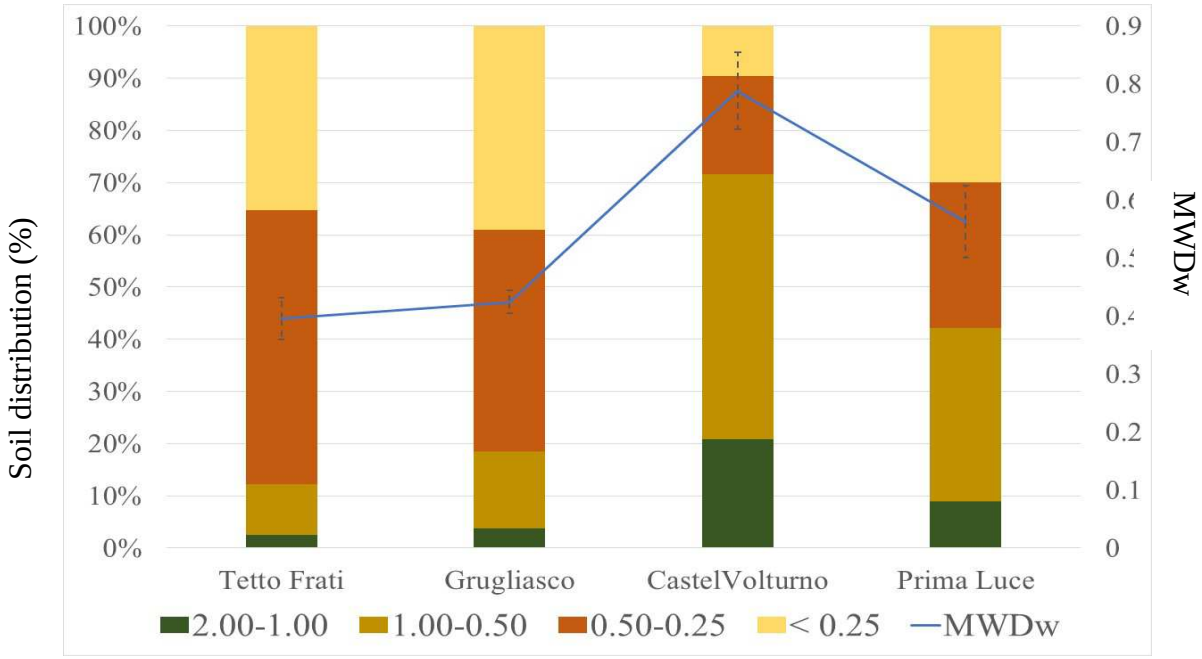

Figure 1. Distribution (%) of water-stable aggregate sizes (mm) and stability index (MWD) in initial soils for different project sites

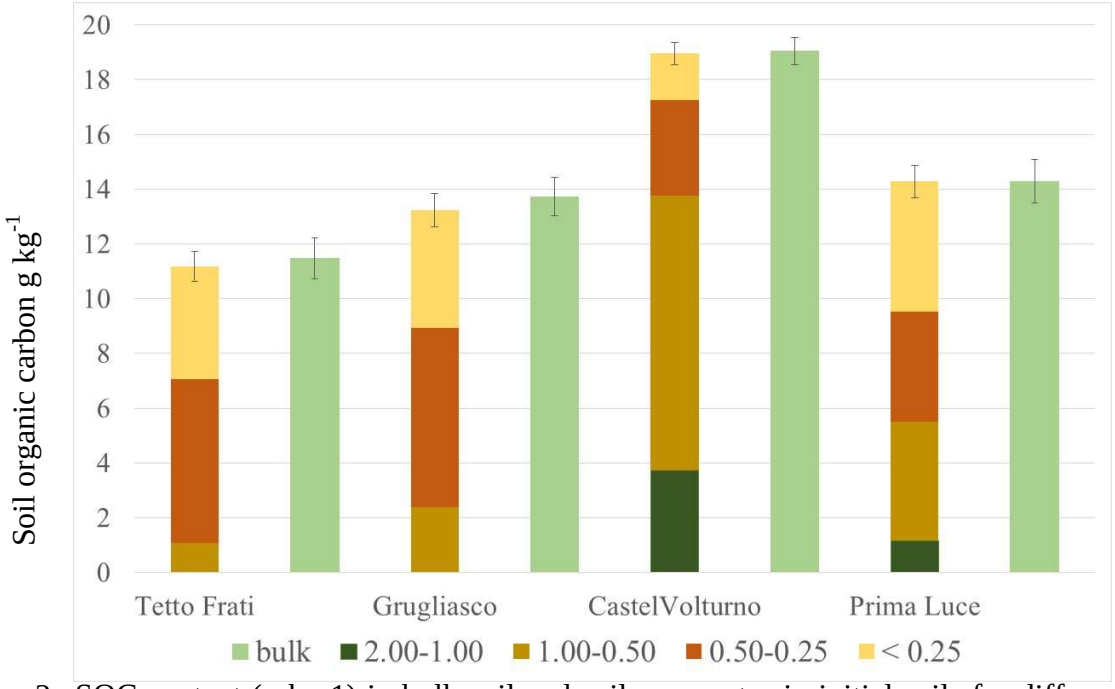

Figure 2a SOC content (g kg-1) in bulk soil and soil aggregates in initial soils for different project sites

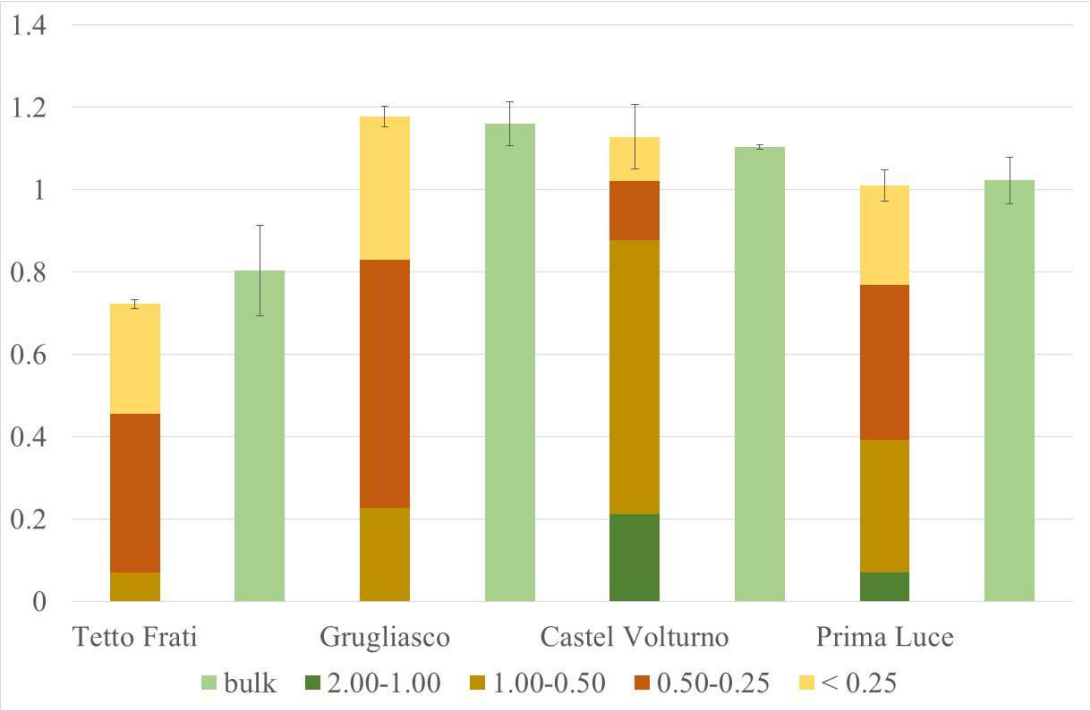

Figure 2b N content (g kg-1) in bulk soil and soil aggregates in initial soils for different project sites

With respect to SOC and N distribution, the lower aggregation capacity of the light textured soils of Tetto Frati and Grugliasco resulted in a null contribution of larger aggregate sizes (2.0-1-0 mm) to TOC and N content, while, the soil from Grugliasco was characterized by lower C/N ratio (Fig. 2a, b).

- off line THM-GC-MS

The total ion chromatograms (TIC) derived from the thermochemolysis of initials soil samples from project sites of Tetto Frati, Grugliasco and Castel Volturno are shown in Figure 3 while the compounds identified in the pyrograms are listed in Table 1. The thermochemolysis released more than hundred recognizable different molecules, which were identified as methyl ethers and esters of natural compounds (Fig.3 and Tab. 1). The majority of these compounds originated from higher plants and microbial by-products and was represented by lignin components, fatty acids, aliphatic biopolymers, hydrocarbons and alcohols. The large yield of THM GC-MS products enabled a feasible quantitative determination of the organic compounds (Tab. 2).

The lignin monomers released by the field plots are inherited from the structural components which build up the lignified tissues of herbaceous plants. The specific compounds have been determined by the main fragmentation pattern (Tab. 1) and were associated to the current symbols used to distinguish the different structural units: P p-hydroxyphenyl, G guaiacyl (3-methoxy, 4-hydroxyphenyl), and S syringyl (3,5-dimethoxy, 4-hydroxyphenyl). The lignin molecules found in initial soil samples (Tab. 1) indicated the presence of, both, fresh decaying plant residues and that of microbial processed organic materials. The latter derivatives included the oxidized products of both di- and tri-methoxy phenylpropane molecules, with the aldehydic (G4, S4), ketonic (G5, S5) and benzoic-acid (G6, S6) forms as main components. Conversely the concomitant release from the

thermochemolysis of soil samples, of 1-(3,4-dimethoxyphenyl)-1(3)-methoxy-propene (G10/11, G13) and 1-(3,4,5-trimethoxyphenyl)- 1(3)-methoxy-propene (S10/11, S13), as either cis or trans isomers (Tab.2), may be related to the incorporation on SOM of slightly decomposed plant debris. Moreover the identification of the enantiomers of 1-(3,4-dimethoxyphenyl)-1,2,3-trimethoxypropane (G14 and G15) and 1-(3,4,5- trimethoxyphenyl)-1,2,3-trimethoxypropane (S14 and S15), confirmed the persistence of not decomposed lignified plant tissues. The aldehydic and acidic forms of guaiacyl and syringyl structures result from the progressive oxidation of lignin monomers, while the corresponding homologues holding methoxylated side chains are indicative of unaltered lignin components, which retain the propyl ether intermolecular linkages. The Ad/Al index is the ratio of peak areas of acidic structures over those of the corresponding aldehydes (G6/G4, S6/S4), while the Γ index is the ratio of peak areas of acidic structures over the sum of peak areas for the threo/erythro isomers (Γ_G = G6/[G14+G15]; Γ_S = S6/[S14+S15]). Both these indices are considered useful indicators of the bio-oxidative transformation of lignin components. The overall larger values found in the initial samples for the majority of the structural indexes (Tab. 2) indicated the prevalence of decomposed lignin monomers. Among the last eluted lignin monomers, the 3-(4,5-dimethoxyphenyl)-2-propenoic (G18) and the 3-(3,4,5-trimethoxyphenyl)-2-propenoic (S18) acid forms, may have originated from either the side chain oxidation of guaiacyl and siringyl units or from the partial decomposition of aromatic domains of suberin biopolymers in plant tissues.

The various alkyl molecules found in the pyrograms, were mainly composed by aliphatic and alicyclic lipid compounds of plant and microbial origin (Tab.1). The most abundant compounds were the methyl ester of linear fatty acids, dominated by the hexadecanoic and octadecanoic saturated and unsaturated homologues. Notwithstanding the multiple possible origins of the C16 and C18 acids, the predominance of even carbon atoms, indicated the plant waxes as prevalent source of the straight chain aliphatic acids. These compounds may derive from the breakdown of long chain ester as well as from the terminal oxidation of other components such as linear hydrocarbons and aliphatic alcohols. The prevailing role of plant input in soil lipid composition was also suggested by the detection of the C24, C26 and C28 aliphatic alcohols (Tab. 2), which are common components of wax layer of non-lignified tissues. This finding was confirmed by the observed distribution of long-chain hydrocarbons (Tab. 2), marked by the peculiar prevalence of heavier odd-numbered alkanes. The off-line pyrolysis, produced also a notable yield of the methylated form of ω-hydroxy alkanoic acids and alkan-dioic acids (Tabs. 1 and 2).

These molecules are the main constituents of the external protective barriers of fresh and lignified plant tissues, namely cutin and suberin. No clear predominance of particular monomer was revealed by both of these compound classes, which instead showed an almost uniform distribution of even carbon-numbered long chain components (Tab. 1). The 9,16-/10,16-dihydroxyhexadecanoic isomers, and the 9,10 epoxide 18 hydroxy-octadecanoic (Tab. 1) acid were the most abundant representative monomers of mid-chain hydroxyl acids, structural units of plant cuticles, frequently used also as plant biomarkers. The relatively least abundant lipid compounds were the high molecular weight tetra- and pentacyclic triterpenes (Tabs. 1 and 2). The sterol and triterpenol molecules have been tentatively identified as methyl ethers and esters of both methyl/ethyl cholesten-3-ol structures, and of ursane, lupeane and oleanane derivatives that are characteristic lipid components of aerial and root plant tissues.

The contribution of microbial input to soil lipids was shown by the inclusion of various structural components of microbial cells, such as phospho-lipid fatty acids (PLFA) and 2-hydroxy aliphatic acids (Tab. 1). The most representative PLFA monomers were, in order of elution, the 12- and 13-methyl tetradecanoic (iso/anteiso pentadecanoic), the 14- and 15-methyl hexadecanoic (iso/anteiso heptadecanoic) acids and the cyclopropane-(2-hexyl)-octanoic acid (C17 cy FAME), which are common microbial constituents of natural organic matter in soil and sediments .

A relative lower amount of carbohydrates derivatives were found among the pyrolysis products of the field management from Torino soil. This finding has been related to the lower efficiency of off-line pyrolysis techniques to detect carbohydrate units of polysaccharides in complex matrices. The thermal behaviour and pyrolitic rearrangement of poly-hydroxy compounds combined with the basic reaction condition of TMAH reagent solution, are believed to negatively interfere in the release of polysaccharides. However, despite the expected low response of carbohydrates, various methylated forms of mono- and oligo-saccharides components were still found among thermochemolysis products (Tabs. 1, 2). These compounds may be mainly associated to xylans and cellulose moieties of coarse ligno-cellulosic debris of plant residues.

Table1 List of thermochemolysis products[a] released from initial soil samples

RT [b]	Compounds	Type
7.55	Methoxy benzene	Lg P1
8.29	Benzene, 1-ethenyl-4-methoxy	Lg P2
8.44	carbohydrate (m/z 101)	Ps
11.06	Benzaldehyde, 4-methoxy	Lg P4
11.17	1H-indole, 2-methyl	Pr
11.31	2,4,5,6,7-pentamethoxyheptanoic acid ME	Ps
11.5	Benzene, 1-methoxy-4-(1-propenyl)	Lg P
11.83	2,4,5,6,7-pentamethoxyheptanoic acid ME	Ps
12.19	Trimethoxybenzene (m/z 110, 125, 153 168)	Ps
12.85	2,4,5,6,7-pentamethoxyheptanoic acid ME	Ps
12.98	Benzoic acid, 4-methoxy ME	Lg P6
13.18	2,6-Dimethoxyphenol	Ps
13.72	Benzene, 4-ethenyl-1,2-dimethoxy	Lg G2
13.91	1,2,3-Trimethoxybenzene	Lg S1
14.4	Carbohydrate (m/z 88, 101, 143)	Ps
14.55	Carbohydrate (m/z 88, 101, 143)	Ps
15.05	Carbohydrate (m/z 88, 101, 143)	Ps
15.28	Carbohydrate (m/z 88, 101, 143)	Ps
15.73	C8 dioic acid ME	Mic
16.42	Carbohydrate (m/z 88, 101, 143)	Ps
17.24	Benzaldehyde, 3,4-Dimethoxy	Lg G4
17.57	C_{12} FAME	Lp
18.49	C_9 dioic acid ME	Mic
19.45	Ethanone, 1-(3,4-dimethoxyphenyl)	Lg G5

19.98	trans-3-(4-Methoxyphenyl)-3-propenoate	Lg P18
20.1	Benzoic acid, 3,4-dimethoxy ME	Lg G6
20.36	Benzaldehyde, 3,4,5-trimethoxy	Lg S4
20.97	cis-1-methoxy-2-(3,4-dimethoxyphenyl)ethylene	Lg G7
21.28	trans-1-methoxy-2-(3,4-dimethoxyphenyl)ethylene	Lg G8
21.91	C_{14} FAME	Lp
22.51	cis-3-(4-Methoxyphenyl)-3-propenoate	Lg P18
22.92	C_{14} FAME	Lp
23.42	Benzoic acid, 3,4,5-trimethoxy ME	Lg S6
24.49	C_{15} iso FAME	Mic
24.7	C15 ante iso FAME	Mic
24.84	cis-1-(3,4,5-Trimethoxyphenyl)-2-methoxyethylene	Lg S7
25.05	threo/erythro-1-(3,4-Dimethoxyphenyl)-1,2,3-	Lg G14
25.29	trans-1-(3,4,5-Trimethoxyphenyl)-2-methoxyethylene	Lg S8
25.39	threo/erythro-1-(3,4-Dimethoxyphenyl)-1,2,3-	Lg G15
25.41	C_{15} FAME	Lp
25.51	cis-1-(3,4,5-Trimethoxyphenyl)-methoxyprop-1-ene	Lg S10
25.78	trans-1-(3,4,5-Trimethoxyphenyl)-methoxyprop-1-ene	Lg S11
26.95	C_{16} branched FAME	Mic
27.36	$C_{16:1}$ FAME	Lp
27.5	cis-1-(3,4-Dimethoxyphenyl)-1,3-dimethoxy-1-propene	Lg G16
27.66	trans-3-(3,4-Dimethoxyphenyl)-3-propenoate	Lg G18
27.73	threo/erythro-1-(3,4,5-Trimethoxyphenyl)-1,2,3-	Lg S14
27.92	C16 FAME	Lp
27.99	threo/erythro-1-(3,4,5-Trimethoxyphenyl)-1,2,3-	Lg S15
28.84	C_{17} branched FAME	Mic
29.31	C_{17} iso FAME	Mic
29.5	C_{17} anteiso FAME	Mic
29.63	Carbohydrate (m/z 88, 101, 143)	Ps
29.83	*cy* C_{17} FAME	Lp
30.09	C_{18} alcohol methyl ether	Lp
30.18	C_{17} FAME	Lp
30.52	3-(3,4,5-trimethoxyphenyl)-2-propenoic	Lg S18
30.64	trans-1,3-dimethoxy-1-(3,4,5-trimethoxyphenyl)-1-	Lg S16
31.87	$C_{18:1}$ FAME	Lp
31.97	$C_{18:1}$ FAME	Lp
32.4	C_{18} FAME	Lp
33.11	C_{16} wOMe FAME	Bp
33.2	C_{19} branched FAME	Lp
34.26	$C_{19:1}$ FAME	Lp
34.43	C_{20} alcohol OMe	Lp

34.51	C_{19} FAME	Lp
35.09	Carbohydrate (m/z 88, 101, 143)	Ps
35.26	C_{16} dioic acid DIME	Bp
35.92	C_{16} diOMe FAME	Bp
36.26	Isopimaric acid ME	Lp/Dt
36.59	Carbohydrate (m/z 88, 101, 143)	Ps
36.65	C_{20} FAME	Lp
37.07	dehydroabietic acid ME	Lp/Dt
37.21	$C_{18:1}$ωOME FAME	Bp
37.68	Carbohydrate (m/z 88, 101)	Ps
37.92	C_{18} ωOME FAME	Bp
38.43	C_{22} alcohol OMe	Lp
38.85	C18:1, 12,13-epoxy-11-methoxy FAME	Bp
39.64	C_{25} n-alkane	Lp
40.41	C_{22} FAME	Lp
41.04	C_{20} ωOMe FAME	Bp
41.45	C_{18} triOMe FAME	Bp
41.70	C_{26} n-alkane	Lp
42.11	C_{24} alcohol OMe	Lp
42.23	C_{23} FAME	Lp
42.55	C_{22} 2-OMe FAME	Mic
43.24	C_{27} n-alkane	Lp
43.97	C_{24} FAME	Lp
44.58	C_{22} ωOMe FAME	Bp
44.90	C_{28} n-alkane	Lp
45.55	C_{26} alcohol OMe	Lp
45.95	C_{24} 2-OMe FAME	Mic
46.55	C_{29} n-alkane	Lp
47.27	C_{26} FAME	Lp
47.86	C_{24} ωOMe FAME	Bp
48.20	C_{31} n-alkane	Lp
50.38	C_{28} FAME	Lp

[a]Bp, plant biopolyester; Ch, carbohydrates; cy, cyclopropane; DIME, dimethyl ester Lg, lignin; Lp, plant lipids (Dt, diterpenoid); FAME, fatty acid methyl ester; Mic, microbial bioproducts; OMe, methoxy; Pr, proteins;.

[b]RT, retention time (min) in total ion chromatogram.

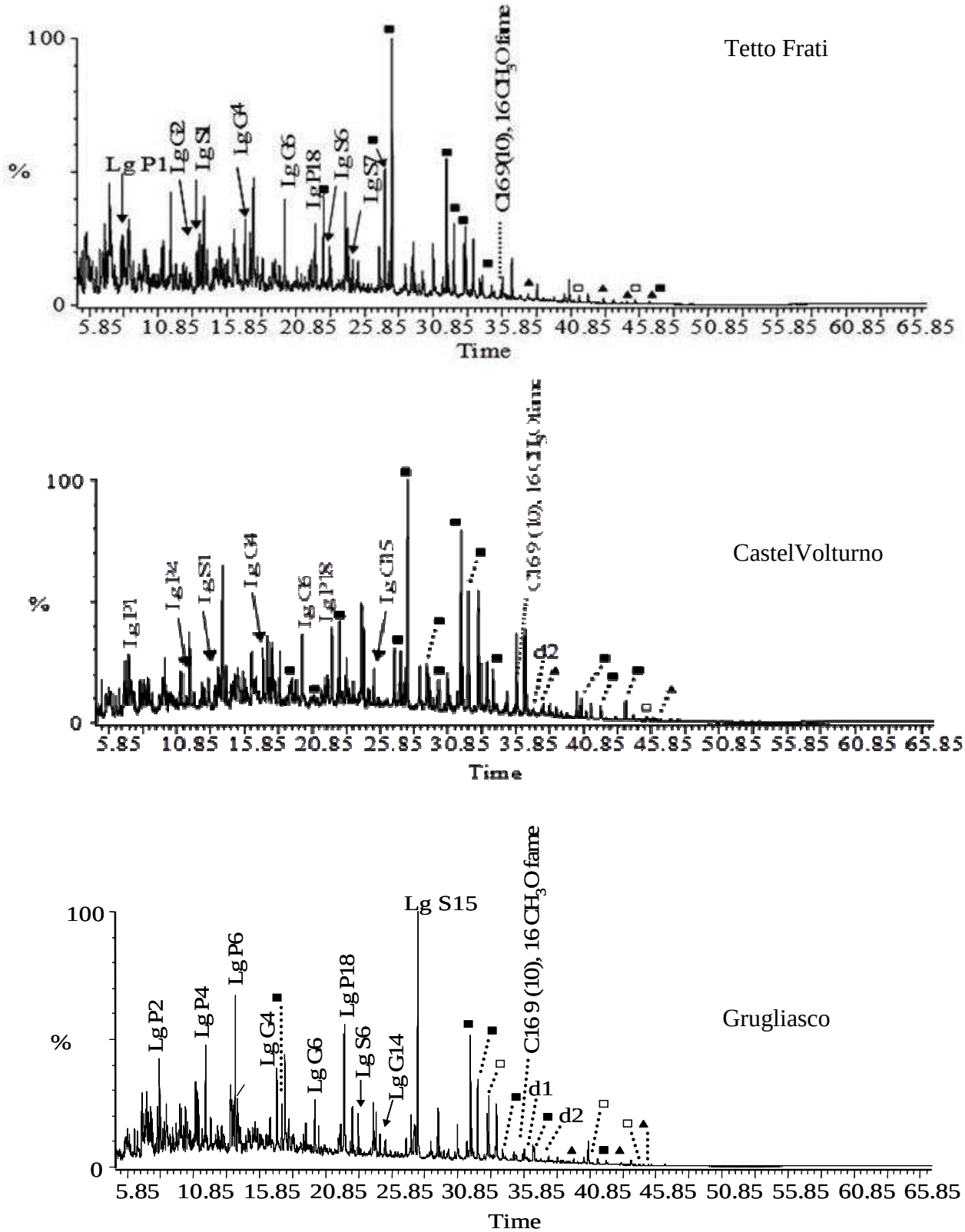

Figure 3 Total ions chromatograms of thermochemolysis products released from initial soils samples: d diterpenoid, Lg Lignin, ■ Fatty acids, ▲ Alkanes, □ Hydroxy acids

Table 2 Composition[a] and yields (µg g^{-1}) of main THM products released from initial soils

Compounds	Tetto Frati	CastelVolturno	Grugliasco
		Lipids	
ω-Hydroxy acids	26.31 (C_{16}÷C_{22})	145.75 (C_{16}÷C_{22})	46.49 (C_{16}÷C_{22})
Mid-chain hydroxy acids	97.23 (C_{16}, C_{18})	136.31 (C_{16}, C_{18})	9.54 (C_{16}, C_{18})
n-Alkanes	8.92 (C_{25}÷C_{31})	14.80 (C_{25}÷C_{31})	5.05 (C_{25}÷C_{31})
Fatty acids	306.38 C_{12}÷C_{28} ($C_{18:1}$)	710.08 C_{12}÷C_{28} ($C_{18:1}$)	183.68 C_{12}÷C_{28} ($C_{18:1}$)
Microbial FAME (%)	6.94	10.70	9.54
Alcohols	19.76 (C_{16}-C_{26})	23.32 (C_{16}-C_{26})	3.94 (C_{16}-C_{26})
Diterpenoid	41.27	105.80	30.22
		Lignin	
Guaiacyl	42.74	59.24	39.50
$(Ad/Al)_G$[b]	2.4	2.3	2.8
(Γ_G)[b]	2.97	2.49	3.35
p-Hydroxyphenyl	26.36	31.98	18.10
Syringyl	37.35	30.74	26.42
$(Ad/Al)_S$[b]	6.5	5.1	1.9
(Γ_S)[b]	3.77	3.57	4.65
Polysaccharides(%)	18.00	20.62	10.70

[a] Total range varying from Ci to Cj; compounds in parentheses are the most dominant homologues; numbers after colon refer to double bond.[b] Structural indexes: $(Ad/Al)_G$=G6/G4; $(Ad/Al)_S$=S6/S4; (Γ_G)=G6/(G14+G15); (Γ_S)=S6/(S14+S15).