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Pyrolysis-Gas Chromatography to Evaluate the Organic Matter Quality of Different Degraded Soil Ecosystems

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1. Introduction

Soil systems are exposed to a variety of environmental stresses of natural and anthropogenic origin, which can potentially affect soil functioning. For this reason, there is growing recognition for the need to develop sensitive indicators of soil quality that reflect the effects of land management on soil and assist land managers in promoting long-term sustainability of terrestrial ecosystems (Bandick & Dick, 1999). Soil organic matter (SOM) providing energy, substrates, and biological diversity necessary to sustain numerous soil functions, has been considered one of the most important soil properties that contributes to soil quality and fertility (Doran & Parkin, 1994; Reeves, 1997).

SOM comprises a range of humic substances (HS) and non humic substances (NHS). Humic substances make up a significant portion of the total organic carbon (TOC) and nitrogen (TN) in soil. They consist of complex polymeric organic compounds of high molecular weights, which are more resistant to decomposition than the NHS. Moreover, humic substances are widely recognized as an important fraction of soil organic matter because they have several fundamental functions: regulation of nutrient availability, linkage with mineral particles and immobilization of toxic compounds (Ceccanti & Garcia, 1994).

On the other hand, NHS contribute to soil ecosystem functionality by providing metabolically labile organic C and N sources, such as low molecular weight aliphatic and aromatic acids, carbohydrates, aminoacids, and their polymeric derivatives such as polypeptides, proteins, polysaccharides, and waxes (Schnitzer, 1991).

In order to understand the temporal dynamics of SOM in soil ecosystems, it is therefore vital to characterize soil organic carbon quantity and quality. Various analytical methodologies, such as infrared (See et al., 2005), ultraviolet–visible (Alberts et al., 2004), nuclear magnetic resonance spectroscopy (Gondar et al., 2005), potentiometric titration, pyrolysis, electrophoresis, acid/alkali fractionation, have been used to study SOM (Ceccanti et al., 2007). These techniques have been used either alone or in combination with traditional fractionation and purification methods of acid/alkali soluble HS. However, these

methodologies may not always reflect native SOM, due to inevitable analytical manipulations and also, sometimes, to a subjective interpretation of the results.

Among these techniques, pyrolysis-gas chromatography (Py-GC) is a technique capable of analysing native SOM, both in bulk soil (without manipulation), and in sodium pyrophosphate extract, considered the favoured solution to extract humic organic matter from soil (García et al., 1992; Masciandaro & Ceccanti, 1999; Clapp & Hayes, 1999). However, some disadvantages exist, since this technique provides information on small molecules but not the overall macromolecular structure (Grandy & Neff, 2008). Nevertheless, Py-GC is a simple and rapid technique which has been used successfully to make a qualitative study of the chemico-structural characteristics of organic matter in soils, sewage sludges (Peruzzi et al., 2011), sediments (Faure et al., 2002), fresh and composted wastes (Garcia et al., 1992; Dignac et al., 2005).

Many papers has been published in the last years on the application of Py-GC to soil analyses (Campo et al., 2011; Sobeih et al., 2008). Nierop et al. (2001) investigated the differences in the chemical composition of SOM within one soil serie from three differently managed fields using a combination of Py-GC/MS and thermally assisted hydrolysis and methylation. The results of this study showed that SOM composition is hardly affected by organic farming compared to conventional management i.e. high tillage intensity and intensive fertilization. Similarly, Marinari et al. (2007a, 2007b) used pyrolytic indices as indicators of SOM quality under organic and conventional management. In these studies has been confirmed that mineral fertilization, showing a relatively large amounts of aliphatic pyrolytic products, contributed to increase the mineralization process.

The Py-GC resulted a promising technique in the assessment of change in soil organic matter composition, even after the application of different types of mulching materials (Ceccanti et al., 2007). In the study of Ceccanti et al. (2007) compost enriched the soil with available organic nutrients, rapidly shown by an increase of pyrolytic aliphatic compounds, while straw application revealed a more stable soil organic matter characterized by a high pyrolytic humification index.

In this chapter the Py-GC technique will be proposed as useful tool to evaluate, on the basis of the chemical structural changes of organic matter, the resistance of degraded soils to different external impacts and rehabilitation practices.

The results obtained from the study of several sites, characterized by local variation of anthropic and natural pressures, will be presented. The investigation has been carried out in the framework of the European project "Indicators and thresholds for desertification, soil quality, and remediation" (INDEX). In this project 12 sites were selected across Europe in order to ensure a broad range of different conditions (natural soil and agro-ecosystems).

The parameters determined by pyrolysis-gas chromatography have been selected as promising indicators of soil degradation and rehabilitation. These parameters were mainly classified as indicator of "State" according to DPSIR (Driving Forces-Pressure-State-Impact-Response) framework of Thematic Strategy for Soil Protection (European Commission, 2006) since making a fingerprint of soil organic matter, they represent the evolution state of organic matter in different climatic and management situation.

2. Methodologies

2.1 Pyrolysis-gas chromatography

Py-GC technique is based on a rapid decomposition of organic matter under a controlled high flash of temperature. A gas chromatograph is used for the separation and quantification of pyrolytic fragments (pyro-chromatogram) originating from organic matter decomposition. 50 micrograms of a representative soil sample, air-dried and ground <100 mesh (bulk soil) or few ml of soil sodium pyrophosphate extract (0.1M) was introduced into pyrolysis quarz microtubes in a CDS Pyroprobe 190.

The textural characteristics of soil can affect the reliability of the Py-GC technique, in particular in degraded soils, for this reason the method can be adapted in order to remove the inert part (for example sand and gross silt) which is not involved in the linkage with the humic substances, in other words, the soil organic matter has to be concentrated. Moreover, for highly organic soils (organic matter > 5%), where a great part of organic matter is linked to quick microbial metabolism, the extraction of humic matter (e.g. sodium pyrophosphate) give more reliable information on native soil organic matter. The Py-GC instrument consisted of a platinum coil probe and a quartz sample holder. Pyrolysis was carried out at 800 °C for 10 s, with a heating rate of 10 °C ms^{-1} (nominal conditions). The probe was coupled directly to a Carlo Erba 6000 gas chromatograph with a flame ionization detector (FID). Chromatographic conditions were as follows: a 3 m x 6 mm, 80/100 mesh, SA 1422 (Supelco inc) POROPAK Q packed column, the temperature program was 60 °C, increasing to 240 °C by 8 °C min^{-1}.

Pyrograms were interpreted by quantification of seven peaks corresponding to the major volatile pyrolytic fragments (Ceccanti et al., 1986): acetic acid (K), acetonitrile (E_1), benzene (B), toluene (E_3), pyrrole (O), furfural (N), and phenol (Y). The retention time (minutes) of the mentioned seven peaks (as standard) are the following: E1, 20.2, K, 23.1, B, 27.8, O, 30.9, E3, 34.7, N, 38.9, Y, 55.6 (Scheme 1). Acetonitrile (E_1) is derived from the pyrolysis of aminoacids, proteins, and microbial cells. Furfural (N), is mostly derived from carbohydates, ligno-cellulosic materials, proteins and other aliphatic organic compounds (Bracewell & Robertson, 1984), indicating the presence of rapidly metabolisable organic substances. Acetic acid (K) is preferentially derived from pyrolysis of lipids, fats, and waxes, cellulose, carbohydrates (Bracewell & Robertson, 1984) and represents relatively less-degraded ligno-cellulose material (Sollins et al., 1996; Buurman et al., 2007). Phenol (Y) is derived from amino acids, tannins and fresh or condensed (humic) lignocellulosic structures (van Bergen et al., 1998; Lobe et al., 2002). Benzene (B) and toluene (E_3) are basically derived from condensed aromatic structures of stable (humified) organic matter, particularly for benzene, since toluene must come from rings with aliphatic chains, albeit short. Pyrrole (O) is derived from nitrogenated compounds such as nucleic acids, proteins, microbial cells and condensed humic structures (Bracewell & Robertson, 1984).

Peak areas were normalized, so that the area under each peak referred to the percentage of the total of the selected seven peaks (relative abundances).

The alphabetic code used was conventional and has already been used in previous papers on natural soils (Ceccanti et al., 1986; Alcaniz et al., 1984). Peak purity of the most important fragments had previously been checked by coupling the same chromatographic system to a mass-detector under the same operating conditions.

Ratios of the relative abundances of the peaks were determined (Ceccanti et al., 1986, 2007) as follows:

N/O: mineralization index of labile soil organic matter. This index expresses the ratio between furfural (N), which is the pyrolytic product arising from polysaccharides, and pyrrole (O), which derives from nitrogenated compounds, humified organic matter, and microbial cells. The higher the ratio, the lower the mineralization of the organic matter, that means a high concentration of polysaccharides may be present.

O/Y: mineralization index of more-stable OM. The higher the ratio, the higher the mineralization of the organic matter, because pyrrole (O) derives from nitrogenated compounds and microbial cells and phenol (Y) mostly derives from polyphenolic compounds.
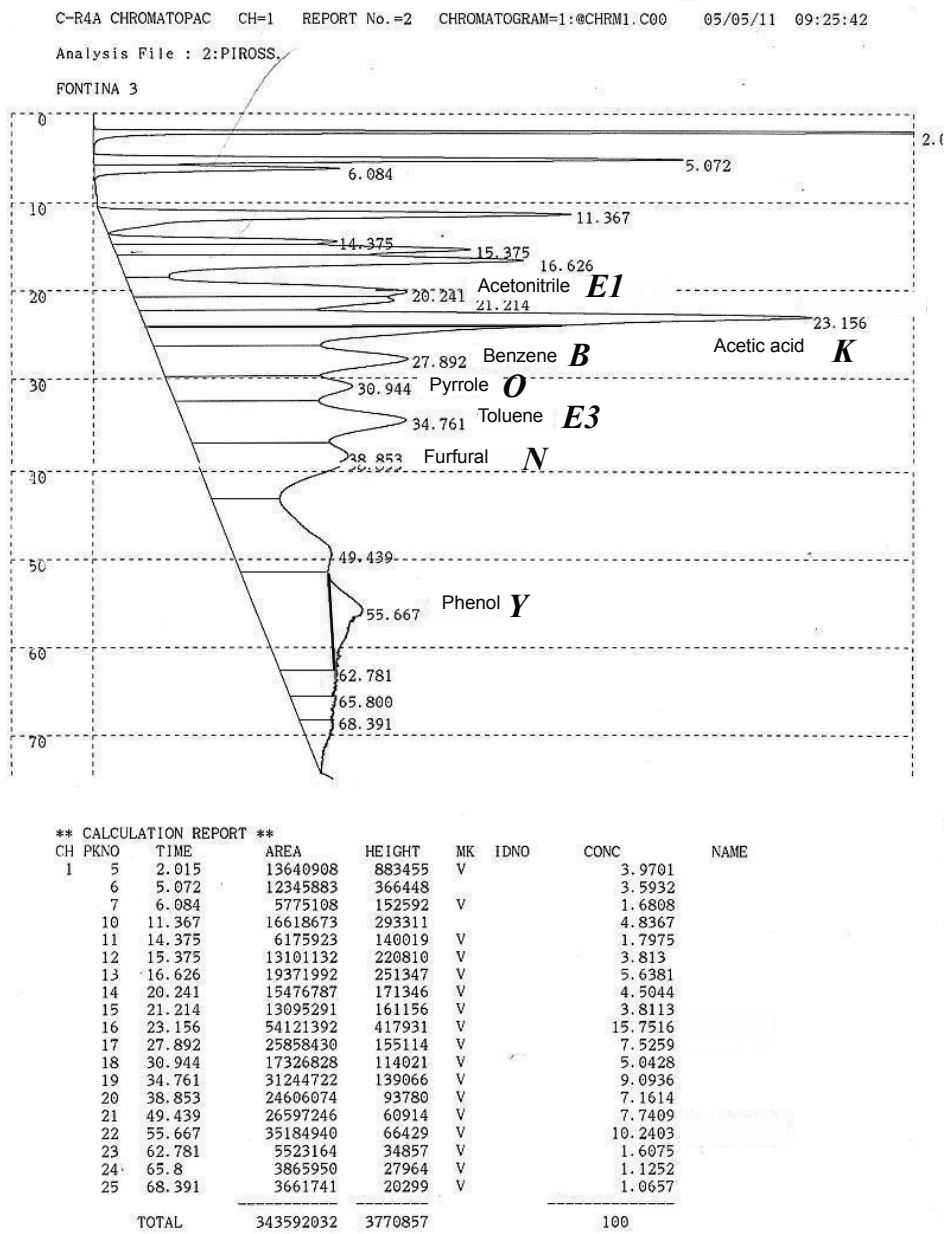
Scheme 1. Pyrogram of sample with time retention and area of each fragment: Acetonitrile (E1), Acetic acid (K), Benzene (B), Pyrrole (O), Toluene (E3), Furfural (N), Phenol (Y).

B/E_3: humification index. The higher the ratio, the higher the condensation of the organic matter, because benzene (B) derives mostly from the pyrolytic degradation of condensed aromatic structures, while toluene (E_3) comes from aromatic uncondensed rings with aliphatic chains.

AL/AR (Aliphatic/Aromatic compounds): index of ''energetic reservoir''. It expresses the ratio between the sum of aliphatic products (acetic acid K, furfural N, and acetonitrile E_1) and the sum of aromatic compounds (benzene B, toluene E_3, pyrrole O and phenol Y). This index could indicate an ''energetic reservoir'' especially in extremely poor soils since it evaluates the labile and stable parts of HS.

In addition, a numeric index of similarity (Sij) between the relative abundances (I) of the homologous peaks (k) in two pyro chromatograms (i and j), was calculated using the following expression: $Sij = (\sum(Ii/Ij)k)/n$ with $Ii < Ij$, and n is the number of peaks (Ceccanti et al., 1986).

The index of similarity Sij compares a pair of pyrograms without discriminating peaks. The index varies in the range of 0–1: the higher the value, the greater the similarity. However, three conventional levels, very high (>0.85), high (0.75-0.85), middle (0.70–0.75), and low (0.60–0.70) have been suggested for the characterization of heterogeneous materials such as SOM (Ceccanti et al., 1986) and compost (Ceccanti & Garcia, 1994; Ceccanti et al., 2007).

2.2 Humic substances extraction

Soil organic matter comprises a range of humified, non-humified, and biologically active compounds, including readily decomposable materials, plant litter and roots, and dead and living organisms. The humified compounds, representing the most microbially recalcitrant and thus stable reservoir of organic carbon in soil may be more appropriate than the labile organic matter compounds in the definition of "soil quality", they can, in fact, be considered a sort of recorder of the soil's history (Masciandaro & Ceccanti, 1999; Piccolo et al., 1996).

Humic substances based on solubility under acidic or alkaline conditions can be fractionated as following: humin, the insoluble fraction of humic substances, humic acid (HA), the fraction soluble under alkaline conditions but not acidic conditions (generally pH <2), and fulvic acid (FA), the fraction soluble under all pH conditions.

Due to the low solubility and complex chemical structure of these compounds, soil extraction and purification are essential steps to study humic substances (Ceccanti et al., 1978). Sodium pyrophosphate is the favoured solution used to extract organic matter from soil, and, neutral conditions permit to extract the greater part of humic carbon, maintaining the functional properties of soil (Masciandaro & Ceccanti,1999).

The extraction of humic substances from the soils were carried out by mechanical shaking of the soil samples with 0.1 M $Na_4P_2O_7$ solution at pH 7.1 for 24 h at 37 °C in a soil/solution ratio of 1/10. The extracts were centrifuged at 24 000 x g for 20 min and filtered on a 0.22 μm Millipore membrane. Identical volumes of these extracts were dialysed in Visking tubes with distilled water. After dialysis, extracts were concentrated by ultrafiltration (AMICON PM10 cut-off membrane) and brought to the volume that extracts had prior to dialysis (Nannipieri et al., 1983; Masciandaro et al., 1997).

3. Case studies

Five case studies have been selected in order to validate the pyrolysis gas-chromatography methodology as useful tool to evaluate chemico-structural changes of soil organic matter: i) two natural soil ecosystems (Catena) with different density of natural plant cover (natural soil), one located in the south of Spain and the other in the south of Italy, ii) two sites under organic (biological) and mineral (conventional) management, one in Cyprus Island and one in the centre of Italy, and iii) a degraded soil undergone to rehabilitation practice with different amount of organic matter (restored soils).

Each soil sample has been collected at 0-15 cm depth and the pyrolysis gas-cromatography has been carried out both in the bulk soil, and in sodium pyrophosphate extract of each sample.

3.1 Natural soil

3.1.1 Spanish catena

A site in Santomera area (Murcia region, Spain) characterized by three different gradual degradation states, related to different natural plant cover establishment and soil slope, has been sampled (sandy loam, USDA classification): i) a natural soil with a 50-60% of vegetation cover of *Pinus halepensis* (high plant density, **Forest**) and a soil slope of 5%, ii) a partially degraded soil with a 20-30% of vegetation cover (autochthonous xerophytic shrub) (low plant density, **Shrub**) and slope of 5%, and iii) a bare soil with only a 5-10% of vegetation cover (scant plant density, **Bare**) and slope of 10%.

The main difference among the soil samples was presented by the percentage of toluene (E_3), the significant increase along the catena ecosystem (Bare > Shrub > Forest) (Table 1), indicated the prevalence of organic matter less-humified in Bare and Shrub with respect to Forest soil, since E_3 comes from aromatic uncondensed rings with aliphatic chains.

In agreement with this result, lower values of benzene (B) and phenol (Y), which derived principally from humified stable organic matter, were found in Bare soil with respect to Forest and Shrub soil, suggesting the reduction of condensed aromatic structure of stable humified organic matter (Masciandaro & Ceccanti, 1999) in consequence of a fast metabolism, involving probably the native humic matter.

bulk soil	E_1%	K%	B%	O%	E_3%	N%	Y%
Forest	11,7	11,7	18,0	22,9	15,4	17,6	2,60
Shrub	11,5	11,3	18,5	20,3	18,5	17,1	2,72
Bare	13,2	10,0	17,4	21,1	19,8	16,8	1,66

Table 1. Relative abundances (%) of main pyrolytic peaks (acetonitrile E_1, acetic acid K, benzene B, pyrrole O, toluene E_3, furfural N, phenol Y) in bulk soils.

Soil differentiation was better shown by the pyrolytic indexes of humification (B/E_3) and mineralization (O/Y and N/O). The humification index B/E_3 which represents the structural condition of a 'condensed aromatic nucleus' of humic substances, increased with the increase of the intensity of vegetation (Forest > Shrub > Bare) (Figure 1), indicating the importance of plant cover in the storage of more stable humus and in the reduction of degradation process (Vancampenhout et al., 2010).

In Bare soil, the scant vegetation seemed unable to protect the soil organic matter from mineralization, as confirmed by the higher value of O/Y (Figure 1). However, the N/O mineralization index, that gave an evidence of the existence of an easily mineralizable fraction of organic matter, did not show many differences among the soils (Figure 1), suggesting that the labile organic matter was less influenced.

Percentages of stable benzene-derived aromatic (AR = B + E_3 + O + Y) and mineralizable aliphatic (AL = E_1 + K + N) structures were very similar in all the three soils (Figure 1). This would indicate that, despite the highest mineralization occurred in Bare soil, the catena ecosystem was self-regulating and still preserved a significative amount of microbial resistant humic substances (Ceccanti & Masciandaro, 2003).
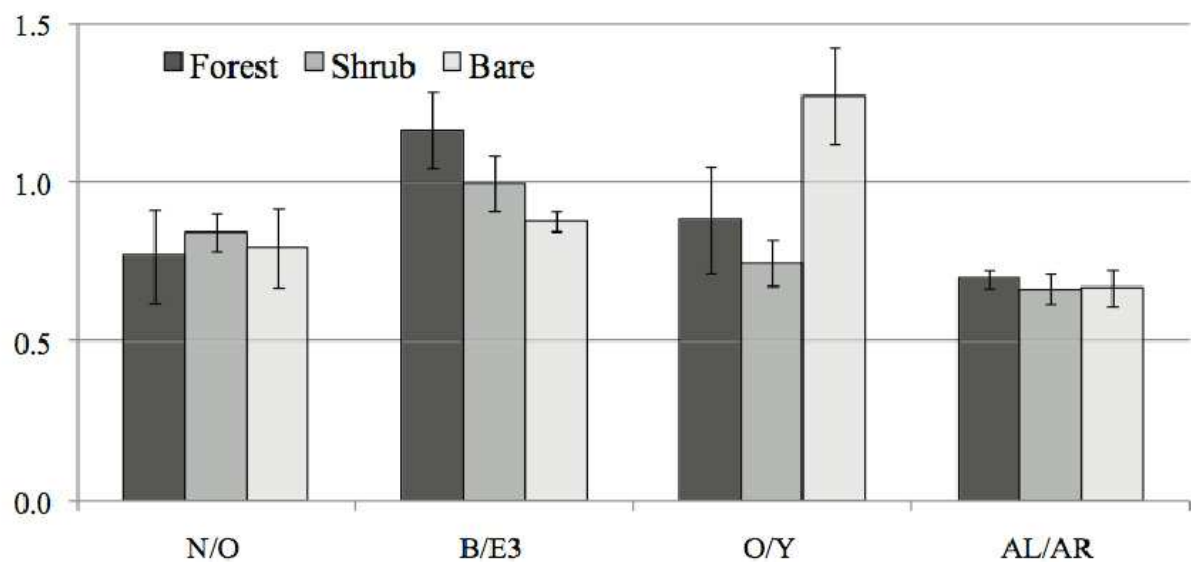

Fig. 1. Indices of mineralization (furfural/pyrrole N/O, pyrrole/phenol O/Y), humification (benzene/toluene B/E_3), and aliphatic/aromatic (AL/AR) ratios (AL=E_1+K+N, AR=B+O+E_3+Y) (mean of three replicates ± standard deviation) in bulk soils The values of O/Y have been divided by 10.

This sort of natural balance in the Catena ecosystem, was confirmed by the high indexes of similarity observed among the three soil samples and, in particular, between Forest and Shrub (Table 2).

bulk soil	Shrub	Bare
Forest	0,938	0,857
Shrub		0,883

Table 2. Pyrolitic index of similarity in the bulk soils.

In the soil extract, the chemical and structural information is amplified by the fact that unlike the bulk soil, the humic fraction is more concentrated and pure and free from interfering substances. In this case no great differences were generally observed among the soils, both in the content of pyrolytic fragments (Table 3) and calculated indices (Figure 2). However, the higher value of N/O found in Bare with respect to Forest soil, suggested that

the mineralization of organic matter, hypothesized on the basis of mineralization index O/Y of bulk soil, involved the labile fraction of humic matter. The high similarity indexes observed among the bulk soils (Table 2) were maintained also among the humic extracts (Table 4), underlining a better homogeneity considering the humic part of the organic matter.

Soil extract	E_1%	K%	B%	O%	E_3%	N%	Y%
Forest	8,26	8,53	16,6	19,7	27,2	13,4	6,31
Shrub	8,43	7,48	14,9	18,8	26,3	15,1	9,31
Bare	6,98	10,0	18,2	17,1	27,4	15,4	4,86

Table 3. Relative abundances (%) of main pyrolytic peaks (acetonitrile E_1, acetic acid K, benzene B, pyrrole O, toluene E_3, furfural N, phenol Y) in soil extracts.
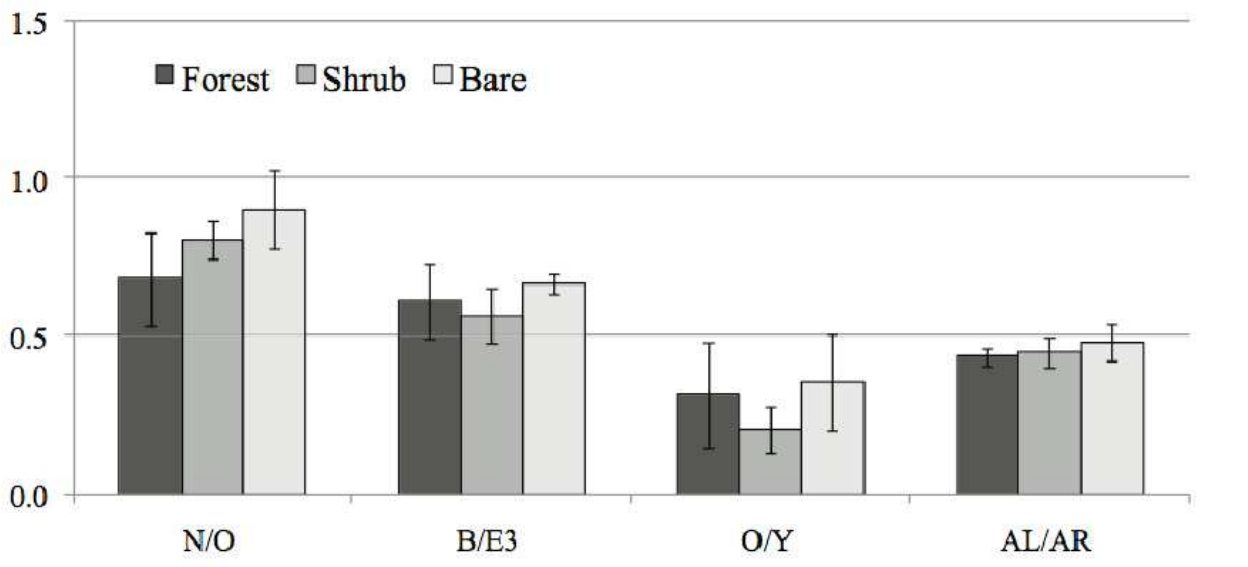

Fig. 2. Indices of mineralization (furfural/pyrrole N/O, pyrrole/phenol O/Y), humification (benzene/toluene B/E_3), and aliphatic/aromatic (AL/AR) ratios (AL=E_1+K+N, AR=B+O+E_3+Y) (mean of three replicates ± standard deviation) in soil extracts. The values of O/Y have been divided by 10.

soil extract	Shrub	Bare
Forest	0,872	0,888
Shrub		0,820

Table 4. Pyrolitic index of similarity in the soil extracts.

3.1.2 Italian catena

A natural catena similar to the Spanish one, were studied in the South of Italy (Basilicata region) (sandy, clay, loam, USDA classification).

Three different gradual degradation states, related to different natural plant cover establishment and soil slope, has been sampled: i) a natural soil with a 60-70% of vegetation cover of *Quercus pubescens* Willd (high plant density, **Forest**) and a soil slope of 15%, ii) a

partially degraded soil with a 20-30% of vegetation cover, consisting in autochthonous xerophytic shrub and grass (low plant density, **Shrub**) and slope of 6%, and iii) an eroded soil without plant cover (no plant, **Bare**) and slope of 20%.

In this Italian natural Catena, differently from the previous, the main difference among the three soils concerned the pyrrole (O) and particularly phenol (Y) fragments (Table 5). An increase of phenol in Bare suggested a slower soil metabolism that slightly affected the dinamic of stable humic matter, probably due to the lack of vegetation cover. The simultaneous increase in pyrrole (O) and phenol (Y) fragments in each soil, indicated the same level of mineralization processes of more stable soil organic matter in the three different degraded soils.

However, as already found for Spanish catena, there was a decrease in humification index with the decrease of vegetal cover (Figure 3). This trend, together with the lower value of AL/AR and N/O in Bare soil, suggested that a great mineralization process interesting the labile part of the organic matter affected this environment.

A lower similarity was, in fact, found among the soils and in particular between Forest and Bare (Table 6) soils.

bulk soil	E_1%	K%	B%	O%	E_3%	N%	Y%
Forest	16,0	9,53	16,1	10,6	24,5	10,0	14,2
Shrub	11,9	11,2	14,6	11,2	23,0	11,8	16,2
Bare	8,75	11,2	11,0	14,5	24,6	8,09	21,9

Table 5. Relative abundances (%) of main pyrolytic peaks (acetonitrile E_1, acetic acid K, benzene B, pyrrole O, toluene E_3, furfural N, phenol Y) in bulk soils.
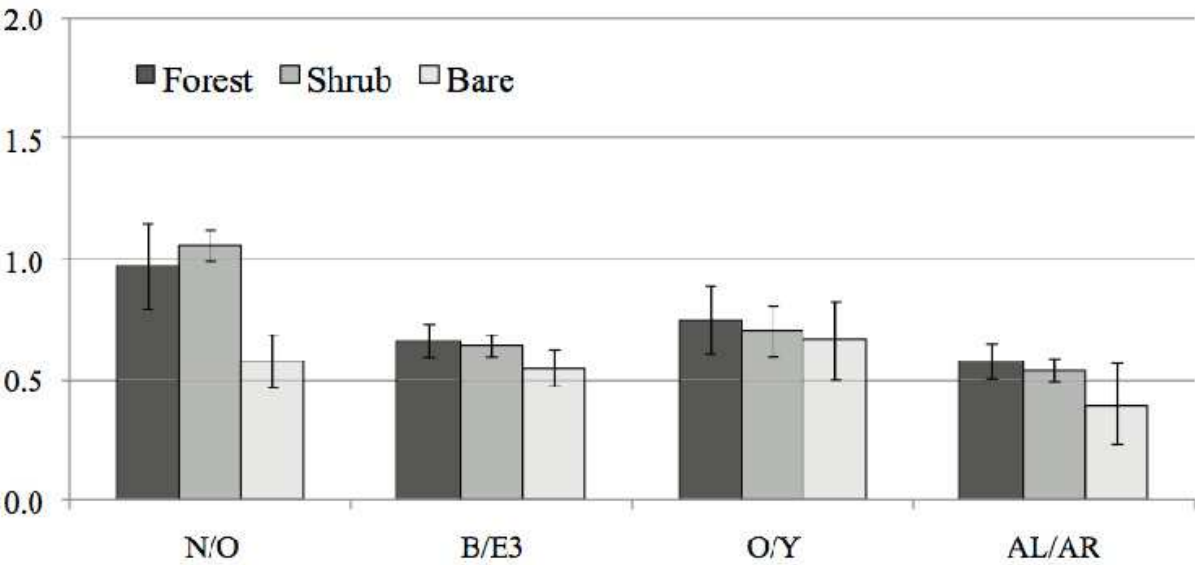

Fig. 3. Indices of mineralization (furfural/pyrrole N/O, pyrrole/phenol O/Y), humification (benzene/toluene B/E_3), and aliphatic/aromatic (AL/AR) ratios (AL=E_1+K+N, AR=B+O+E_3+Y) (mean of three replicates ± standard deviation) in bulk soils.

bulksoil	Shrub	Bare
Forest	0,845	0,752
Shrub		0,801

Table 6. Pyrolitic index of similarity in the bulk soils

Soil extract	E1%	K%	B%	O%	E3%	N%	Y%
Forest	8,94	11,4	23,4	9,90	20,0	12,3	13,9
Shrub	13,8	13,9	19,2	12,9	14,2	14,5	11,5
Bare	6,39	16,1	25,7	12,0	22,0	9,55	8,32

Table 7. Relative abundances (%) of main pyrolytic peaks (acetonitrile E_1, acetic acid K, benzene B, pyrrole O, toluene E_3, furfural N, phenol Y) in soil extracts.

In the humic extract, the trend of the mineralization indices O/Y and N/O, highlighted the hard mineralization process that affect bare soil, followed by shrub soil (Figure 4, Table 7).

In this Catena ecosystem, differently from the previous, the scant vegetation and probably other environmental factors such as greater soil slope and Mediterranean climate, with dry hot summer and cold winters with strong storm, make the bare soils more susceptible to erosion processes.

However, the high values of B/E_3 detected in each soil, suggested that this ecosystem is able to preserve the more condensed humic matter.

As already observed for the bulk soils, medium-high similarity indices were found among the humic extracts of the different situations (Table 8). Therefore, both Italian and Spanish Catena, were more homogeneous considering the humic part of the organic matter.
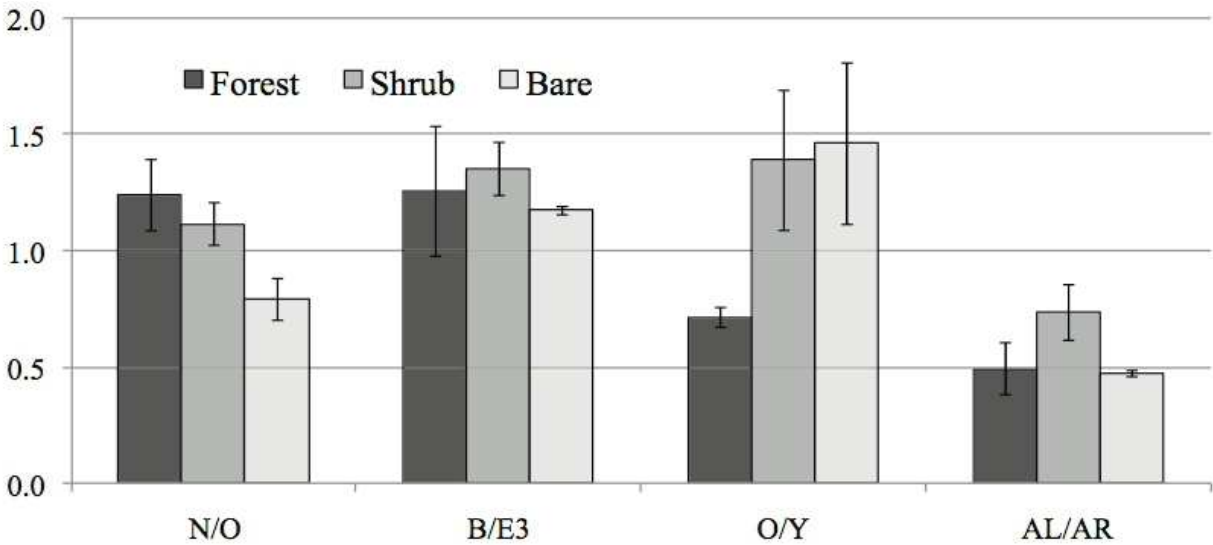

Fig. 4. Indices of mineralization (furfural/pyrrole N/O, pyrrole/phenol O/Y), humification (benzene/toluene B/E_3), and aliphatic/aromatic (AL/AR) ratios (AL=E_1+K+N, AR=B+O+E_3+Y) (mean of three replicates ± standard deviation) in soil extracts.

Soil extract	Shrub	Bare
Forest	0,778	0,778
Shrub		0,718

Table 8. Pyrolitic index of similarity in the soil extracts

3.2 Organic and mineral management

3.2.1 Site in Cyprus Island

Two adjacent fields in Cyprus Island, where one was managed according to biological, and the other according to conventional farming methods, were studied (sandy clay loam soil, USDA classification). In the biological agriculture, the fields were fertilized with 10 t ha^{-1} y^{-1} of commercial manure (in pellets), while in the conventional treatment mineral fertiliser was added at a total rate of 300 kg ha^{-1}.

A clear difference between biological and conventional management was evident in Py-GC fragments analysis (Table 9): a higher abundance of benzene (B) and phenol (Y) characterized the biological treatment, thus indicating the establishment of a humified stable organic matter nucleus. On the other hand, the greater value of toluene (E$_3$) and pyrrole (O) found in conventional treatment highlighted the presence of humified organic matter, especially that in less condensed and pseudo-stable form (Table 9). In addition, the high presence of pyrrole (O) in conventional management suggested the triggering of mineralization processes. In fact, the higher value of O/Y and the lower value of N/O in conventional treatment (Figure 5), highlighted the higher level of mineralization of less mineralizable and easily degradable organic matter, respectively, occurred in this soil. Instead, the impressive degree of index of humification B/E$_3$ found in biological treatment suggested the incorporation of organic matter added to soil addressed the metabolic processes towards the organic matter humification.
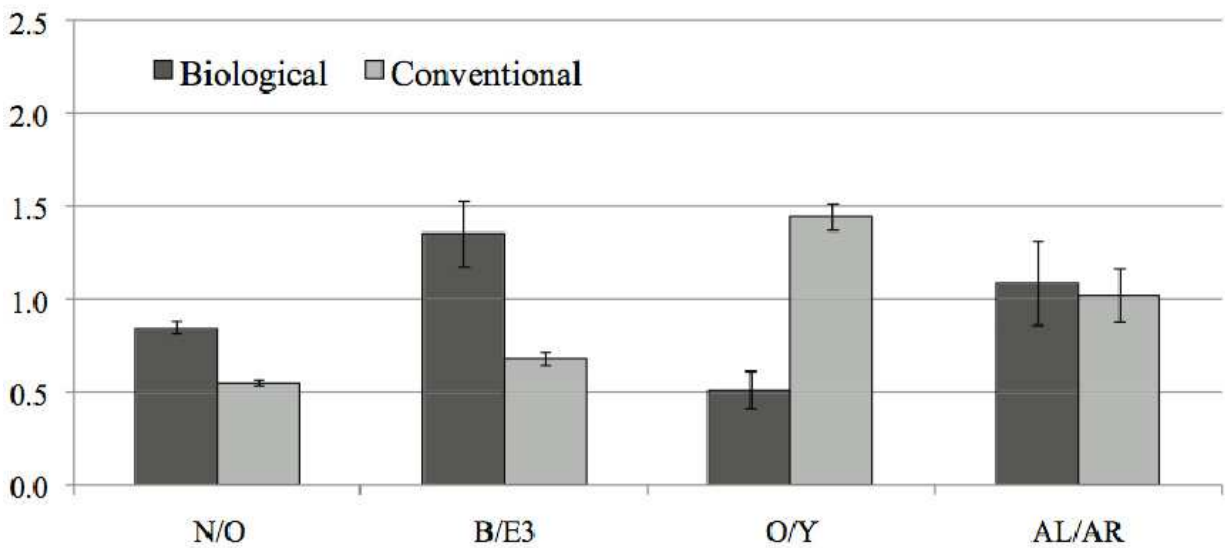

Fig. 5. Indices of mineralization (furfural/pyrrole N/O, pyrrole/phenol O/Y), humification (benzene/toluene B/E$_3$), and aliphatic/aromatic (AL/AR) ratios (AL=E$_1$+K+N, AR=B+O+E$_3$+Y) (mean of three replicates ± standard deviation) in bulk soils.

bulk soil	E_1%	K%	B%	O%	E_3%	N%	Y%
Biological	26,1	20,3	16,8	6,23	12,8	5,29	12,4
Conventional	29,1	15,5	13,0	10,4	19,0	5,75	7,21

Table 9. Relative abundances (%) of main pyrolytic peaks (acetonitrile E_1, acetic acid K, benzene B, pyrrole O, toluene E_3, furfural N, phenol Y) in bulk soils.

The results obtained from the analysis of humic extracts, discriminated more the different influence of the two management practices on soil organic matter quality (Table 10). The effect of compost addition on soil under biological treatments was, in fact, highlighted by the higher level of acetic acid (K) and pyrrole (O) relative abundances with respect to the conventional one, as also found by Ceccanti et al. (2007). The values of these compounds suggested the presence of fresh organic matter and the beginning of a humification process mediated by microbial activity (Peruzzi et al., 2011). The higher relative abundance of toluene (E_3) in conventional site was in agreement with the results of bulk soil pyrolytic fragments, thus showing the presence of less stable humic fraction. The index of mineralization N/O (Figure 6) showed that, in biological treatment, mineralization process of easily degradable organic matter occurred. However, the highest index of humification in biological site, as shown by pyrolytic indices in the bulk soil, suggested that the organic matter added with compost has became part of humic pool of soil (Garcia et al.,1992).

The differences occurred in management practises could be resumed by similarity indices, which clearly expressed the difference between the two treatments both in bulk soil and in pyrophosphate extract (S_{soil}: 0.743, $S_{extract}$: 0.774).
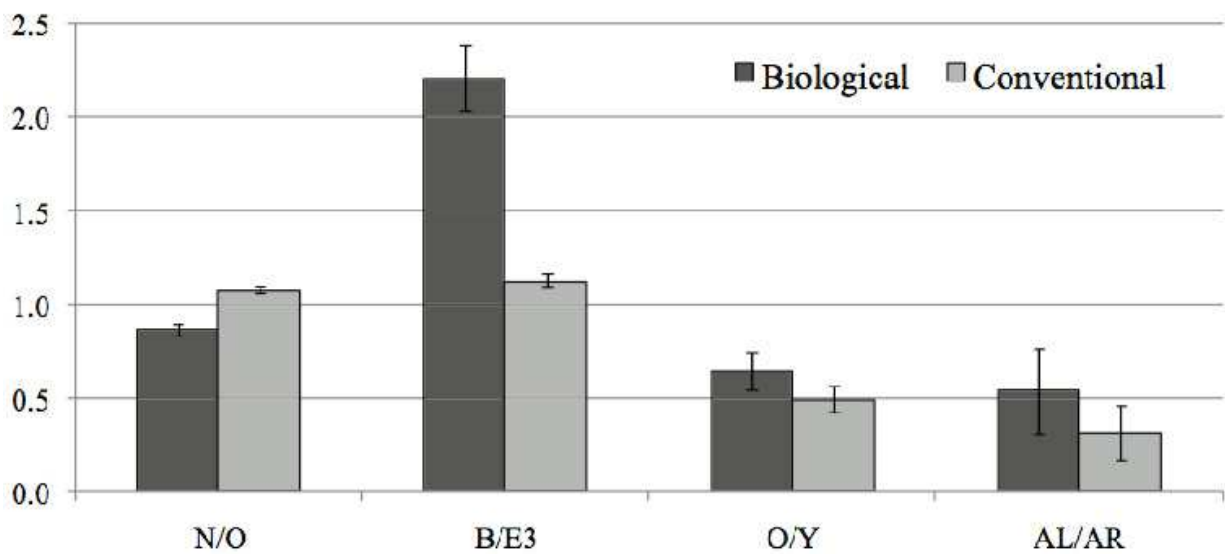

Fig. 6. Indices of mineralization (furfural/pyrrole N/O, pyrrole/phenol O/Y), humification (benzene/toluene B/E_3), and aliphatic/aromatic (AL/AR) ratios (AL=E_1+K+N, AR=B+O+E_3+Y) (mean of three replicates ± standard deviation) in soil extracts.

Soil extract	$E_1\%$	K%	B%	O%	$E_3\%$	N%	Y%
Biological	4,20	17,88	18,9	14,6	8,63	12,6	23,2
Conventional	4,49	6,95	21,5	11,7	19,2	12,4	23,7

Table 10. Relative abundances (%) of main pyrolytic peaks (acetonitrile E_1, acetic acid K, benzene B, pyrrole O, toluene E_3, furfural N, phenol Y) in soil extracts.

3.2.2 Site in Italy

Two adjacent fields in Central Italy (Tuscany region), one managed according to biological, and the other according to conventional farming methods, were also studied. The soil of both fields was classified as sandy clay loam according to the USDA classification. In the biological agricultural soil (biological procedure for five years), green manure (1,5 t ha^{-1} y^{-1}) was applied to the fields, while in the conventional agricultural soil, the fields received mineral fertilizer (0,2 t ha^{-1} y^{-1} ammonium nitrate).

The Italian situation showed fewer differences both in soil and extract, considering the two management practices, with respect to the Cyprus case (Similarity indices S_{soil}: 0.873, $S_{extract}$: 0.870). However, a higher level of benzene (B) and pyrrole (O) characterized biological treatment (Table 11). This result suggested that this kind of soil management promoted: i) the establishment of more humified organic matter, as also suggested by the trend of humification index B/E3, and ii) the stimulation of microbial metabolism driving the mineralization processes of the available organic matter, as suggested by the trend of O/Y and N/O indices (Figure 7). In other words, the biological management stimulated more the process of organic matter turnover, with respect to the conventional one, which on the contrary, started the mineralization of the humified organic matter, as suggested by the highest value of O/Y in the humic extract (Figure 8, Table 12). However, the trend of humification index B/E_3 of the extracts (no difference between conventional and biological treatment), indicating that the conventional treatment did not still affect the stable nucleus of humic fraction.
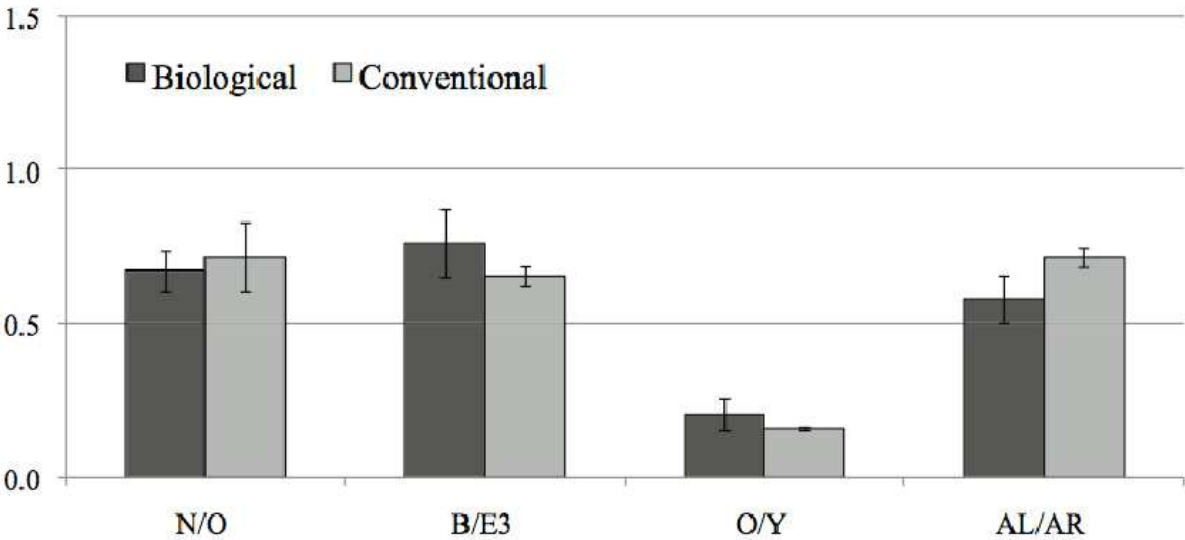

Fig. 7. Indices of mineralization (furfural/pyrrole N/O, pyrrole/phenol O/Y), humification (benzene/toluene B/E_3), and aliphatic/aromatic (AL/AR) ratios (AL=E_1+K+N, AR=B+O+E_3+Y) (mean of three replicates ± standard deviation) in bulk soils. The values of O/Y have been divided by 10.

bulk soil	E_1%	K%	B%	O%	E_3%	N%	Y%
Biological	14,3	11,4	16,8	16,4	22,1	11,0	8,06
Conventional	21,0	11,0	14,3	13,6	21,9	9,70	8,63

Table 11. Relative abundances (%) of main pyrolytic peaks (acetonitrile E_1, acetic acid K, benzene B, pyrrole O, toluene E_3, furfural N, phenol Y) in bulk soils.

Soil extract	E1%	K%	B%	O%	E3%	N%	Y%
Biological	3,38	13,4	21,8	15,6	24,0	13,8	8,02
Conventional	6,00	15,9	22,8	15,8	23,9	11,5	4,10

Table 12. Relative abundances (%) of main pyrolytic peaks (acetonitrile E_1, acetic acid K, benzene B, pyrrole O, toluene E_3, furfural N, phenol Y) in soil extracts.
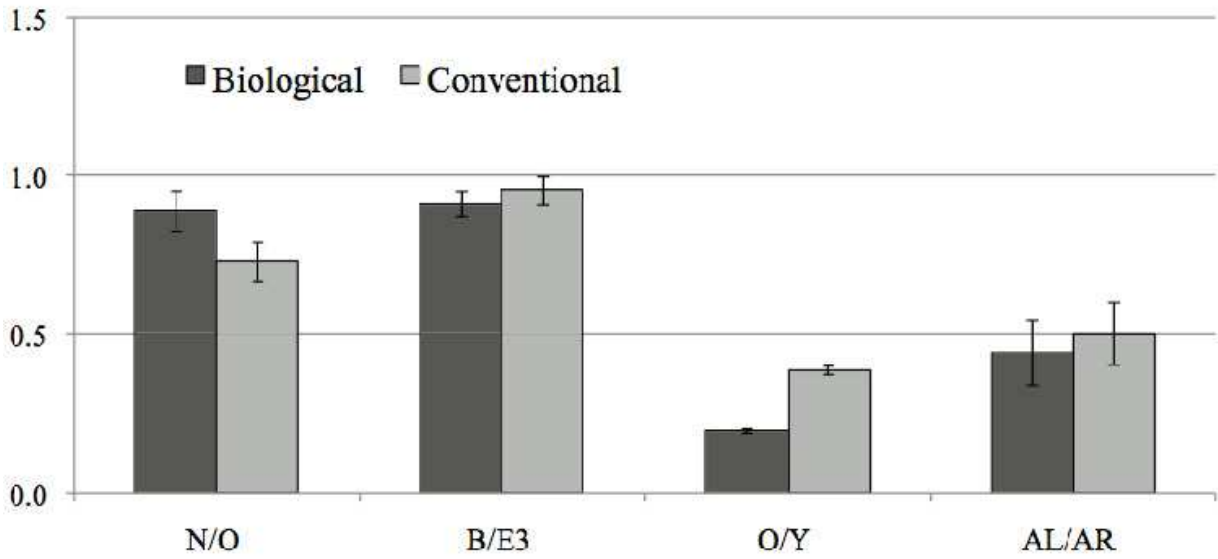

Fig. 8. Indices of mineralization (furfural/pyrrole N/O, pyrrole/phenol O/Y), humification (benzene/toluene B/E_3), and aliphatic/aromatic (AL/AR) ratios (AL=E_1+K+N, AR=B+O+E_3+Y) (mean of three replicates ± standard deviation) in soil extracts. The values of O/Y have been divided by 10.

3.3 Restoration of degraded soils

An experimental field (sandy clay loam soil, USDA classification) in province of Murcia (Spain) was split into plots in which 20 years ago was added a single dose of fresh easily degradable municipal organic waste (MOW) in such dose as to increase the soil organic matter by 0.5 (65t/ha), 1.0 (130 t/ha), 1.5 (200t/ha) and 2.0% (260 t/ha), MOW fraction was incorporated into the top 15 cm of soil using a rotovator. The aim was to restore biochemical and microbial properties and to contrast the erosion through a stimulation of spontaneous establishment of grass cover. One plot without the addition of MOW was used as control (0%). The plots were monitored for three years: following organic amendments an averagely 50-70% plant coverage developed and persisted throughout the experiment until today. The highest plant density were found in 1.5% treatment (80-90%) while only 20-30% plant coverage was developed in the control soil. After 16 years from the treatment the same

vegetated plots have been sampled at 0-15 cm depth in order to assess and investigate the changes occurred in consequence of the vegetal cover establishment.

The input of exogenous OM produced qualitative changes of soil OM both in the bulk soil and in the pyrophosphate extracts, which are still evident after 16 years. Considering the different plots, in fact, there were significant differences in the Py-GC fragments analysis: acetonitrile (E_1) and acetic acid (K) increased with the increase of organic matter added (Table 13), at the same time lower values of pyrrole (O) and phenol (Y) were detected in the same plots. The increase of acetonitrile and pyrrole were probably due to the higher cover of vegetation restored in the plots, which bring to higher quantity of cellulose, lipids and easily degradable compounds released into the soil. This trend was also confirmed by AL/AR index, which increased at the increase of the organic matter application (Figure 9).

The plots treated with the higher quantity of organic matter, also showed higher level of humification index (B/E_3), thus suggesting that after 16 years the humification overrides the mineralization process. As suggested by to the humification index, the N/O ratio showed higher values in the plots with the greater application of organic residues, indicating the presence of more evolved (less mineralizable) organic compounds in the soil. The similarity indices confirmed the role of the MOW in affecting the quality of organic matter (Marinari et al., 2007) (Table 14), in that 1%, 1.5% and 2% treatments resulted more similar between them.

bulk soil	E_1%	K%	B%	O%	E_3%	N%	Y%
Organic matter 0.0%	6,39	3,54	11,8	15,1	24,0	14,0	25,2
Organic matter 0.5%	8,81	6,57	12,5	16,9	20,3	16,0	19,5
Organic matter 1.0%	14,4	11,3	15,3	10,6	20,0	10,3	17,4
Organic matter 1.5%	11,9	13,2	14,1	12,7	20,1	14,1	13,9
Organic matter 2.0%	14,6	11,6	14,7	10,7	19,9	12,7	15,7

Table 13. Relative abundances (%) of main pyrolytic peaks (acetonitrile E_1, acetic acid K, benzene B, pyrrole O, toluene E_3, furfural N, phenol Y) in bulk soils.
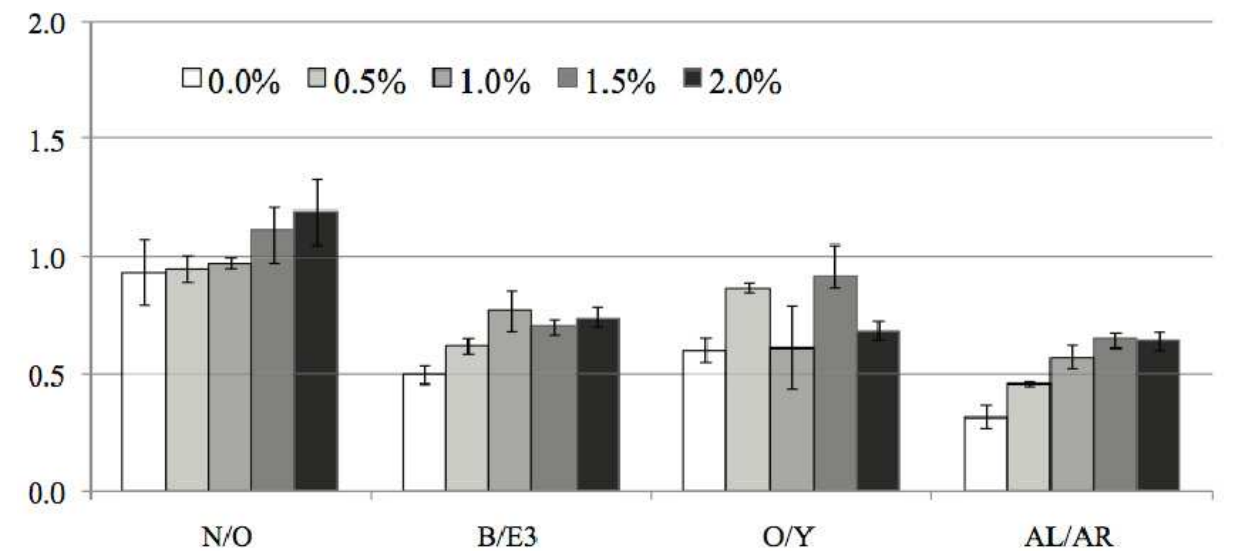

Fig. 9. Indices of mineralization (furfural/pyrrole N/O, pyrrole/phenol O/Y), humification (benzene/toluene, B/E_3), and aliphatic/aromatic (AL/AR) ratios (AL=E_1+K+N, AR=B+O+E_3+Y) (mean of three replicates ± standard deviation) in bulk soils.

bulk soil	0,5%	1%	1,5%	2%
0%	0,800	0,641	0,695	0,660
0,5%		0,736	0,779	0,747
1.0%			0,853	0,945
1,5%				0,898

Table 14. Pyrolitic index of similarity in the bulk soils.

The results of the humic extract highlighted the great difference among the 1.5% and the others treatments (Table 15,16 and Figure 10). In fact, the higher vegetal cover in 1.5% plots probably promoted the enrichment of soil in labile and less stable fraction of organic matter, which resulted the more sensitive to degradation process, as suggested by the trend of N/O and O/Y. As a consequence, in 1.5% treatment a lower value of B/E_3 was observed, probably due to the masking of humic matter from fresh materials.

Soil extract	E_1%	K%	B%	O%	E_3%	N%	Y%
Organic matter 0.0%	3,66	3,94	16,4	13,2	21,2	18,7	22,9
Organic matter 0.5%	2,62	2,40	17,9	12,5	21,5	19,2	24,0
Organic matter 1.0%	3,46	3,77	18,1	12,0	22,5	17,2	23,2
Organic matter 1.5%	7,88	6,05	12,7	14,9	22,2	17,1	19,0
Organic matter 2.0%	6,07	4,93	15,6	12,2	23,6	15,9	21,7

Table 15. Relative abundances (%) of main pyrolytic peaks (acetonitrile E_1, acetic acid K, benzene B, pyrrole O, toluene E_3, furfural N, phenol Y) in soil extracts.
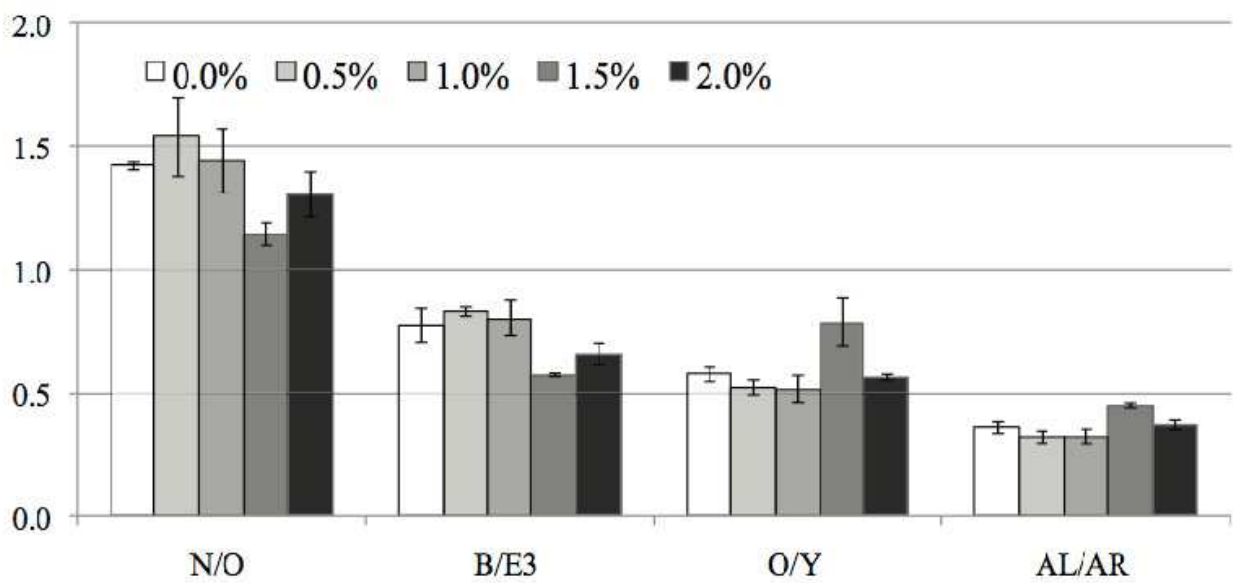

Fig. 10. Indices of mineralization (furfural/pyrrole N/O, pyrrole/phenol O/Y), humification (benzene/toluene B/E_3), and aliphatic/aromatic (AL/AR) ratios (AL=E_1+K+N, AR=B+O+E_3+Y) (mean of three replicates ± standard deviation) in soil extracts. The values of O/Y have been divided by 10.

Soil extract	0,5%	1%	1,5%	2%
0%	0,872	0,938	0,781	0,888
0,5%		0,88	0,703	0,773
1.0%			0,766	0,855
1,5%				0,852

Table 16. Pyrolitic index of similarity in the soil extracts.

4. Statistical considerations

In order to understand the importance of PY-GC technique in describing the chemico-structural and the functionality of soil organic matter, the principal component analysis (PCA) was used. PCA is a multivariate statistical data analysis technique that reduces a set of raw data into a number of principal components that retain the most variance within the original data to identify possible patterns or clusters between objects and variables.

In order to develop wider conclusions about the state of soil organic matter a data set containing information about different ecosystems was used. 12 sites were selected across Europe in order to ensure a broad range of different conditions (natural soil and agro-ecosystems) and related pressures: Spain (Abanilla, Santomera, El Aguilucho, Tres Caminos, all in Murcia region) Italy, (Castelfalfi and Alberese in Tuscany region, Pantanello in Basilicata region), Germany (Puch, in Bavaria region) and Hungary (Gödöllö). All selected sites were characterized by local variation of anthropogenic and natural pressures additionally to the variation that can be found at the European scale. The details of all sites are listed in Table 17. The selected sites may be grouped onto two different types:

Type one: Catenae. Pressures and soil degradation change along a gradient, for example along a slope with differing steepness or along a landscape with a sequence of plant cover. The selected catenae imply a variation of pressures that is mainly of natural origin. Samples are taken from different locations along the catenae. The sites were situated in Spain (Abanilla, Carcavo, Santomera) and Hungary (Godollo) and are described in Table 17.

Site	Description	Treatment Pression	Experiment
Puch (Germany)	Agricultural field under normal cultivation	Tillage/ Plant Cover	Management variation
	Field kept bare of vegetation with plowing		
	Very limited vegetation maintained		
Abanilla (Spain)	Bare	Erosion	Catena
	Esparto grass		
	High coverage		
	Medium coverage		
	Sludge added 0%	Organic matter	Management variation
	Sludge added 5%		
	Sludge added 10%		
	Sludge added 15%		
	Sludge added 20%		

Site	Description	Treatment Pression	Experiment
Carcavo (Spain)	Abandoned	Erosion/ Exposition	Catena
	Degraded		
	Vegetated with pines		
	North slope		
	South slope		
El Aguilucho (Spain)	Control	Plant Cover/ Erosion/ Organic Matter	Management variation
	Pine		
	Pine + Organic Matter (OM)		
	Terrace + Pine		
	TP+ mycorrize		
	TPm + OM		
	TP+ soil mycorrize		
	TP + OM		
	TPMs + OM		
Santomera (Spain)	Bare	Plant Cover/ Erosion	Management variation/ Catena
	Forest		
	Shrub		
	Removed forest		
	Forest		
Tres Caminos (Spain)	Compost	Plant Cover/ Organic matter	Management variation
	Humus enzymes		
	Control		
	Reforestation		
	Reforestation + mycorrize		
	Seed		
	Sewage Sludge		
Gödöllö (Hungary)	Accumulated	Erosion	Catena
	High erosion		
	Medium erosion		
	No erosion		
Pantanello (Italy)	Organic agriculture	Organic matter/ Agrochemicals	Management variation
	Conventional agriculture		
Castelfalfi (Italy)	Disturbed forest	Animal pressure/ Organic matter/ Erosion	Management variation
	Undisturbed forest		
Alberese (Italy)	Organic agriculture	Organic matter/ Agrochemicals	Management variation
	Conventional agriculture		

Table 17. Site description

Type two: Variation of management. Different types of treatments have been applied to plots at the same site some time ago. These treatments may either represent an anthropogenic pressure, which promotes soil degradation or a remediation action, from which lasting effects may be expected. Type two also contains adjacent sites under different agricultural and forest management, where for instance organic farming may be compared to common farming practice or fenced off forest areas may be compared to the rest of the forest with a high animal population density. The sites were situated in Germany (Puch), Spain (Abanilla, El Aguilucho, Santomera, Tres Caminos) and Italy (Pantanello, Castelfalfi and Alberese) and are described in Table 17.

The PCA of the data set about bulk soils indicated 80% of the data variance as being contained in the first three components (Table 18). PC1, PC2 and PC3 account for 31.0%, 27.9% and 22.0% of the total variance, respectively. PC1 was closely associated with B, O, Y and both indices of mineralization (O/Y and N/O), while PC2 was linked with K, E_3, humification index B/E_3 and AL/AR index. PC3 was associated with E_1 and N. As expected, the indices of mineralization had an opposite behaviour: when O/Y increase, N/O decrease and vice versa, being involved in pseudo-stable and labile organic matter metabolism, respectively. In PC2, the high correlation occurred between humification index B/E_3 and AL/AR index, suggested that both labile and stable organic matter affected soil humification processes.

	Factor 1	Factor 2	Factor 3
Acetonitrile (E_1 soil)	0.027	0.139	*0.899**
Acetic acid (K soil)	0.072	*0.875**	0.190
Benzene (B soil)	*0.730**	-0.014	0.285
Pyrrole (O soil)	*0.804**	0.020	-0.483
Toluene (E_3soil)	0.071	*-0.819**	0.367
Furfural (N soil)	-0.257	0.136	*-0.872**
Phenol (Ysoil)	*-0.786**	-0.283	-0.352
Mineralization index (N/O soil)	*-0.788**	0.031	-0.321
Humification index (B/E_3 soil)	0.290	0.868*	-0.188
Mineralization index (O/Y soil)	*0.895**	0.012	-0.165
Energetic reservoir index (AL/AR soil)	-0.137	*0.871**	0.375
Explained .Variance	3.398	3.068	2.482
Total Proportionality	30.89%	27.89%	22.57%

Table 18. Analysis of principal components on bulk soil pyrolytic fragments and indices. *Variables with component loading used to read the PC.

The results of PCA of the data set about soil extracts were similar to those about bulk soil, with few important exceptions concerning pyrolytic fragments and index related to the more stable part of humic matter. In the same way of bulk soil, PCA of the extract isolated three principal components (PC) (total variance explained: 70.6%). The 1st PC (29.9 % of the total variance) included E_3 and the same parameters of bulk soil (O, Y, O/Y and N/O) with the exception of B that, in this case, was included in the 3nd PC (17.1 % of the total variance) together with humification index (B/E_3). Therefore, differently from the bulk soil, the humification index resulted independent from the other indices, suggesting the importance of the PY-GC on the pyrophosphate extract to investigate the condensed aromatic nucleus of humic substances. The 2nd PC(23.7% of the total variance) was associated, as for bulk soil, with K and AL/AR index (Table 19).

	Factor 1	Factor 2	Factor 3
Acetonitrile (E_1 extract)	0.074	-0.479	0.203
Acetic acid (K extract)	-0.089	*-0.886**	-0.225
Benzene (B extract)	-0.256	0.358	*-0.804**
Pyrrole (O extract)	*-0.742**	0.260	0.109
Toluene (E_3 extract)	*-0.749**	0.352	0.207
Furfural (N extract)	0.271	0.319	0.494
Phenol (Y extract)	*0.771**	0.485	0.134
Mineralization index (N/O extract)	*0.742**	0.056	0.346
Humification index (B/E_3 extract)	0.355	-0.001	*-0.833**
Mineralization index (O/Y extract)	*-0.859**	-0.102	-0.074
Energetic reservoir index (AL/AR extract)	0.083	*-0.960**	0.067
Explained. Variance	3.279	2.604	1.879
Total Proportionality	29.80%	23.67%	17.08%

Table 19. Analysis of principal components on humic extract pyrolytic fragments and indices. *Variables with component loading used to read the PC.

5. Conclusions

The results obtained by PY-GC carried out on the bulk soil and humic extract of several European sites, characterized by different management practices, permitted to draw these final considerations:

i.   Mineralization indices N/O and O/Y resulted the pyrolytic parameters that better discriminate among the different soils, being involved in the dynamics of labile organic matter, linked to microbial metabolism, and pseudo-stable matter, related to the humic fraction.

ii. The pyrolysis on the humic extract often evidenced information hidden by the analysis carried out on the bulk soil. In particular, the pyrolysis on the pyrophosphate extract permitted to obtain clearer information on the condensed aromatic nucleus of humic substances.

iii. Even if the analysis on soil as such (bulk) and on soil humic extracts gave each specific information on the quality of the organic matter; the scientific details obtained from the pyrophosphate extract agreed with those resulted analysing the bulk soil, meaning that this kind of extract does not alter the chemical properties of the soil organic matter.

Finally, it is possible to conclude that the PY-GC can be used as a quick and general reproducible technique to make a qualitative study of the chemico-structural characteristics of soil organic matter turnover related to biological activity (bulk soil), and of the more stable humic fraction (pyrophosphate extract) in soils under different anthropogenic and natural pressures.

Further researches could involve the study of the link between the chemico-structural properties of the humic fraction and their capability to protect enzyme in biologically active form.

6. Acknowledgment

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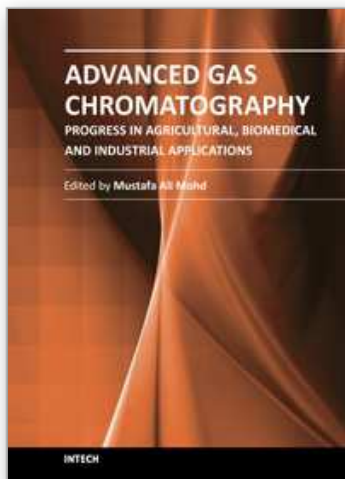
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Progress in agricultural, biomedical and industrial applications' is a compilation of recent advances and developments in gas chromatography and its applications. The chapters cover various aspects of applications ranging from basic biological, biomedical applications to industrial applications. Book chapters analyze new developments in chromatographic columns, microextraction techniques, derivatisation techniques and pyrolysis techniques. The book also includes several aspects of basic chromatography techniques and is suitable for both young and advanced chromatographers. It includes some new developments in chromatography such as multidimensional chromatography, inverse chromatography and some discussions on two-dimensional chromatography. The topics covered include analysis of volatiles, toxicants, indoor air, petroleum hydrocarbons, organometallic compounds and natural products. The chapters were written by experts from various fields and clearly assisted by simple diagrams and tables. This book is highly recommended for chemists as well as non-chemists working in gas chromatography.

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