RESEARCH ARTICLE

Soil organic matter quality in three Mediterranean environments (a first barrier against desertification in Europe)

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Abstract

The aim of this study was to establish the effect of parent material, climate conditions and vegetation type on soil organic matter (SOM) quality in different Mediterranean environments as an essential step for assessing soil stability; ecosystems in the first barrier against desertification in the western Mediterranean region. Thirty-six samples were taken from the organic-mineral horizon of unreclaimed soils with calcareous, acid metamorphic and calcareous/volcanic substratums; and from beneath three vegetation types (natural forests, reforested forests and scrublands) in areas of significant ecological value. The humus fractions and soil respiratory activity of bulk soil samples were studied, and some structural features of the humic acids extracted were characterized by elemental analysis and visible and infrared spectroscopies. Results indicate that SOM in the surface horizons of the calcareous and calcareous/volcanic areas showed higher biogeochemical transformation, where microbial synthesis in humic acids formation, including condensation mechanisms, prevails. In contrast, the acid metamorphic area showed the greatest differences, and would be considered more fragile in terms of organic matter stability to changes in the biogeochemical system. The results also indicate some differences due to the type of vegetation on soil humus chemistry. Under reforested forests of *Pinus* in an edaphic acid environment, humic acids showed a marked aliphatic character, displayed higher 2920 cm^{-1} IR band, well defined typical lignin patterns, and higher E_4/E_6 ratio, i.e., accumulation of inherited macromolecular substances. The SOM beneath scrubland and natural *Quercus* forests may be said to be more decomposed (active breakdown of biomacromolecules) than beneath *Pinus*, humic acids exhibited higher aromaticity and were associated with accumulation of newly formed perylenequinonic chromophors of fungal origin, i.e., was at more advanced and complex stages of humification.

Keywords: Soil humic acids, humification mechanisms, reforested forests, Mediterranean mountains

1. Introduction

Soils in the Mediterranean region are extremely vulnerable, especially those in high-mountain and semi-arid or arid areas. This is particularly important because one of the main environmental problems facing the regions bordering the Mediterranean sea, and large areas of the European Mediterranean region, is the threat of land degradation and progressive desertification (Rubio and Recatalá, 2006; Hill *et al.*, 2008). Mediterranean mountain ecosystems, many of them well conserved, are of great value in slowing desertification because of the generalized presence in the region of intensively cultivated lands. Human pressure has also modified land use in mountain environments with different production systems and exploitation of resources for decades, resulting in unknown ecological effects.

Study of the nature of SOM, and their relationship with vegetation type and other environmental factors, is vitally important in areas with high risk of degradation and desertification, such as the Mediterranean region, where this information is of great importance for evaluating soil stability, and thus, the fragility of their ecosystems. Knowledge about SOM is essential to be able to assess the consequences of modifying some highly specific ecological and pedological conditions in the vulnerable ecosystems in the Mediterranean region, in a context where climatic conditions become more extreme.

Organic matter is essential in the stabilization of soil aggregates, water infiltration, conservation of biogeochemical nutrient cycles, and erosion control. The soil organic carbon pool is an important part of the global carbon pool and plays an essential role in global climate change and ecosystem stability (Li *et al.*, 2013). The susceptibility of soils to erosion in Mediterranean mountainous areas largely depends on upper mineral soil horizon properties, which are controlled by or related to humus form development (Sevink *et al.*, 1998). Low SOM concentrations in arid or semiarid environments can lead to progressive

degradation of their quality and productivity (Caravaca *et al.*, 2002). Soils having organic matter with higher humification degree are more stable and more resistant to the onset of degradation. Hence, because SOM quality is also of vital importance from the perspective of its environmental functions, as reported in several articles in the area under study (e.g., Aranda *et al.*, 2011; Miralles *et al.*, 2012), and coinciding with Almendros *et al.* (2005), the interest of assessing SOM quality rather than its total concentration must be underlined.

Some studies related to SOM quality have been done in the southernmost zone of Mediterranean Europe (Oyonarte *et al.*, 1994; Delgado *et al.*, 2007; Miralles *et al.*, 2007; Miralles *et al.*, 2012). However, some aspects as important as the effect of the parent material, climate conditions and vegetation, including intensive reforestation, have been poorly studied. According to Castro *et al.* (2004) and Ruiz-Sinoga *et al.* (2012), the development of suitable strategies for identifying those silvicultural and management practices in the Mediterranean basin remain a major issue. Additional information on general factors affecting soil C sequestration or stabilization in the area under study would be of great interest.
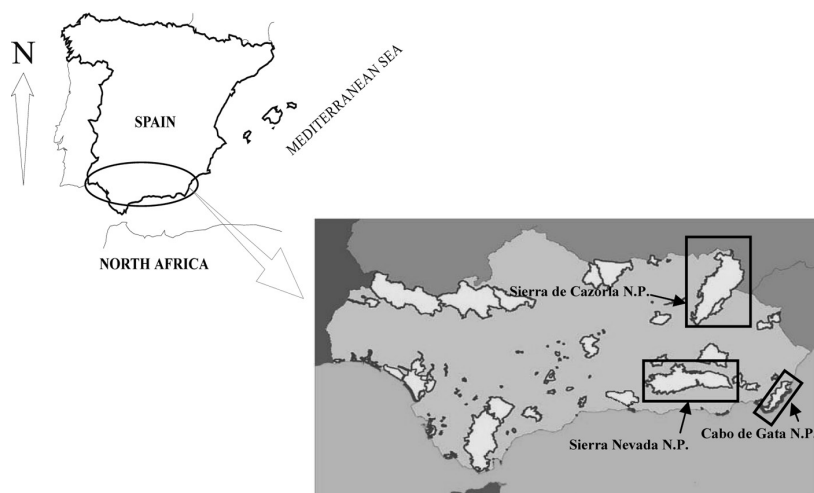
The protected natural areas in the south western-most part of the Mediterranean basin studied in this work have many natural environments containing native and managed soils. All of them provide Europe´s first barrier against the advance of desertification from North Africa. In this context, the general aim of this work was to study the characteristics of organic matter and the prevailing humic substances formation processes in soils from three natural areas of high ecological value in severe risk of degradation. The primary goal was to analyse the influence of the parent material and vegetation type on the evolution and quality of the organic fraction in these fragile Mediterranean environments. The secondary goal was to verify the current influence of intensive reforestations with pine trees before becoming natural parks.

2. Materials and Methods

2.1. Site description and soil sampling

The soil samples studied were taken from 36 uncultivated soils (Ah horizons) in three areas of high ecological value in SE Spain (Figure 1). The Sierra Nevada National Park, (SN): 12 soils over an acid metamorphic substratum, xeric soil moisture regime, and mesic soil temperature regime. The Cazorla-Segura-Las Villas Natural Park, (SC): 12 soils over a calcareous substratum, xeric soil moisture regime, and mesic soil temperature regime. And the Cabo de Gata-Níjar Natural Park, (CG): 12 soils over calcareous and volcanic substrata, arid soil moisture regime, and thermic soil temperature regime. The climate is typically Mediterranean in all three zones, with strong seasonal contrasts. Mean annual rainfall was 595 mm in SN, 866 mm in SC and 240 mm in CG. The mean annual temperatures were 9.8ºC in SN, 11.1ºC in SC and 17.8ºC in CG.

Figure 1. Distribution of protected natural areas in Andalusia (Southern Spain) and location of sampling areas: Sierra Nevada National Park (SN samples), Sierra de Cazorla Natural Park (SC samples) and Cabo de Gata Natural Park (CG samples).

The soils to be sampled within each sampling zone were chosen from three vegetation types: monospecific natural forests (B), about 50-60-year-old monospecific pine tree reforestations (R) and high-diversity scrublands (M). While this criterion was applied in SC and SN, in CG, only soils from scrubland were sampled as there was no other type of vegetation. The most relevant field data are shown in Table 1. Soil profiles were sampled and described, taking well conserved plant communities (coverage of 75% or more) across each natural park as references. Five subsamples were taken from surface horizons distributed randomly in a 10 m^2 area around the location of the profile described. These field samples were mixed to form a single composite or bulk sample, which were then analysed in the laboratory. The mean sampling thickness for the Ah horizons was 16 cm (SN samples), 20 cm (SC samples) and 22 cm (CG samples), respectively.

Table 1. Climatic and field general data of the soils from Mediterranean ecosystems (SE Spain)

Soil	Altitude (m a.s.l.)	MAR (mm)	MAT (ºC)	Soil type *	Vegetation type	Geological substrate
SN-1	1800	603.3	9.6	Typic Haploxeroll	Quercus forest (*Quercus pyrenaica*)	Schists
SN-2	1850	614.9	9.3	Lithic Haploxerept	Pinus forest (*Pinus sylvestris*)	Schists
SN-3	1500	533.5	11.5	Typic Haploxerept	Quercus forest (*Quercus pyrenaica*)	Schists
SN-4	2700	812.8	3.8	Lithic Eutrocryept	Scrubland (*Genista versicolor, Juniperus hemisphaerica*)	Schists
SN-5	1600	556.8	10.9	Typic Dystroxerept	Scrubland (*Ulex sp., Cistus sp.*)	Schists
SN-6	1500	533.5	11.5	Typic Haploxerept	Quercus forest (*Quercus rotundifolia*)	Schists
SN-7	1320	491.6	12.6	Typic Haploxerept	Pinus forest (*Pinus sylvestris*)	Schists
SN-8	1800	603.3	9.6	Lithic Dystroxerept	Scrubland (*Adenocarpus decorticans, Genista sp.*)	Schists
SN-9	2000	649.9	8.3	Typic Dystroxerept	Pinus forest (*Pinus sylvestris*)	Schists
SN-10	1650	568.4	10.5	Typic Haploxerept	Pinus forest (*Pinus sylvestris*)	Schists
SN-11	1820	608.0	9.5	Typic Dystroxerept	Scrubland (*Genista versicolor, Adenocarpus decorticans*)	Schists
SN-12	1620	561.4	10.7	Entic Ultic Haploxeroll	Quercus forest (*Quercus rotundifolia*)	Schists
SC-1	1100	970.6	12.6	Lithic Xerorthent	Pinus forest (*Pinus pinaster*)	Limestones
SC-2	1400	1128.3	10.5	Typic Haploxeroll	Quercus forest (*Quercus faginea*)	Calcareous sandstone
SC-3	1250	1049.5	11.5	Lithic Ultic Haploxeroll	Quercus forest (*Quercus rotundifolia*)	Limestones
SC-4	1650	1259.7	8.8	Lithic Haploxerept	Scrubland (*Erinacea anthyllis, Crataegus monogyna*)	Limestones
SC-5	1500	1180.9	9.8	Lithic Haploxeroll	Scrubland (*Ulex sp., Retama sphaerocarpa, Thymus sp.*)	Calcareous Schists
SC-6	1150	996.9	12.2	Typic Haploxerept	Pinus forest (*Pinus pinaster*)	Marls
SC-7	700	760.5	15.4	Typic Haploxerept	Pinus forest (*Pinus pinaster*)	Clays
SC-8	1590	634.0	9.9	Lithic Haploxerept	Quercus forest (*Quercus rotundifolia*)	Dolomites
SC-9	1780	558.0	8.7	Lithic Haploxeroll	Scrubland (*Erinacea anthyllis, Berberis hispanica*)	Limestones
SC-10	1550	756.0	10.1	Typic Haploxeroll	Pinus forest (*Pinus pinaster*)	Dolomites
SC-11	1200	459.0	12.4	Lithic Haploxerept	Scrubland (*Juniperus thurifera, Ulex sp.*)	Limestones/dolomites
SC-12	1410	634.0	11.0	Lithic Haploxeroll	Quercus forest (*Quercus rotundifolia*)	Limestones
CG-1	150	189.0	18.8	Lithic Xeric Haplocambid	Scrubland (*Stipa tenacissima*)	Andesite
CG-2	100	252.0	18.8	Lithic Haplocambid	Scrubland (*Periploca angustifolia, Maytenus europaeus, Chamaerops humilis*)	Andesite
CG-3	60	189.0	18.8	Lithic Haplocambid	Scrubland (*Chamaerops humilis, Rhamnus lycioides, Pistacia lentiscus*)	Andesite
CG-4	3	169.1	18.8	Typic Torripsamment	Scrubland (*Ziziphus lotus, Withania frutescens, Rhamnus lycioides*)	Sand dune
CG-5	40	252.0	18.8	Lithic Torriorthent	Scrubland (*Stipa tenacissima*)	Reef limestone
CG-6	135	290.0	18.6	Xeric Haplocambid	Scrubland (*Ulex parviflorus, Rosmarinus officinalis*)	Andesite/Dacite
CG-7	250	271.6	16.8	Lithic Torriorthent	Scrubland (*Stipa tenacissima*)	Sandstone/Limestone
CG-8	200	252.0	16.8	Xeric Torriorthent	Scrubland (*Anthylis cytisoides*)	Andesite/Dacite
CG-9	170	252.0	16.8	Lithic Haplocambid	Scrubland (*Thymus sp., Helianthemun sp.*)	Dacite
CG-10	20	252.0	16.8	Xeric Torriorthent	Scrubland (*Thymus sp., Helianthemun sp.*)	Beach-rock
CG-11	200	252.0	16.8	Xeric Torriorthent	Scrubland (*Stipa tenacissima*)	Limestone
CG-12	350	259.0	17.4	Xeric Haplargid	Scrubland (*Chamaerops humilis, Rhamnus lycioides, Pistacia lentiscus*)	Metagranite/Gneiss

Sampling zone: Sierra Nevada National Park (SN); Sierra de Cazorla Natural Park (SC); Cabo de Gata Natural Park (CG); MAR: mean annual rainfall; MAT: mean annual temperature; *SSS, USDA, NRCS (2006).

2.2. Soil analyses

All analytical data for the soil samples analysed refers to the fine-earth fraction (< 2 mm). The procedures used for analysis are outlined by the American Society of Agronomy and Soil Science Society of America (Page *et al*., 1982; Klute, 1986): The organic carbon (OC) content was determined by the Walkley-Black´s method with dichromate oxidation. Total N was measured by the Kjeldhal´s method. The pH (1:2.5, w/v) in distilled water was found by potentiometry. The exchangeable bases were extracted with ammonium acetate solution, buffered to pH 7, and concentrations determined by atomic absorption spectrophotometry (AAS). The free iron oxides were extracted with citrate-dithionite using the Holmgren´s method and determined by AAS. The clay particle-size was determined using the Robinson´s pipette method after elimination of the organic matter with H_2O_2 and dispersion with Na-hexametaphosphate.

The 36 samples were subjected to various analyses to characterize the organic matter present. Fractionation of SOM was done following the IHSS procedure (Swift, 1996). Physical separation of the particulate soil fraction consisting of the not-yet decomposed organic remains (free organic matter) by flotation in 0.1 M H_3PO_4, followed by centrifugation and repeated washing with distilled water before the particulate fraction was dried. Total extractable carbon for humic and non-humic substances was extracted from soil by mechanically shaking the samples with a solution of 0.1 M NaOH and $Na4P_2O_7$ at pH 14 for 24 h at 60°C (1:10 w/v). The extracts were centrifuged and filtered (Millipore 0.45 μm). Separation of the total extractable carbon fraction into humic acids (HA) and fulvic acids (FA) was done by precipitation with H_2SO_4 (1:1 by volume) and purification with polyvinylpolypyrrolidone to eliminate non-humic carbon, respectively. The FA fraction was considered the soluble carbon remaining from the humic extract; and the corresponding HA/FA ratio was found. To obtain the insoluble organic carbon (HUMIN), the residue was treated with 1% Na_2S2O_4 followed by 1M

HCl-HF (1:1) at 60°C, in order to remove free oxides and clay, respectively; next, extractable humin was isolated by extraction with 0.5MNaOH, and any remaining carbon was considered non-extractable organic carbon (NEOC). The amounts of carbon in the above fractions were quantified by wet combustion (Page *et al*., 1982).

Mineral impurities of the HA were eliminated as follows: the remaining humic extract was precipitated with HCl, de-ashed with 1M HCl-HF, redissolved in 0.5M NaOH, and centrifuged at 43,500xg; the dissolved salts were eliminated using cellophane bags by dialysis, and finally freeze-dried. Elemental composition of the HA (ash-free basis) was determined with a Perkin-Elmer 240C microanalyser, and the H/C and O/C atomic ratios were calculated. A Shimadzu UV-240 was used for the visible spectroscopy to find the E_4/E_6 ratio (absorbance to 465 nm and 665 nm of the visible spectra) and the derivatographic spectrometry (valleys near 620 nm, 570 nm and 528 nm), both in 0.2 mg C mL^{-1} solutions of HA in 0.1 M $NaHCO_3$ (Stevenson, 1994). Infrared (IR) spectra were obtained from KBr pellets with a 2 mg sample, using a Shimadzu FTIR-8400 PC Fourier-transform spectrophotometer. The spectra were subjected to a digital procedure for resolution enhancement based on the subtraction of the raw spectrum from a positive multiple of its second derivative (Rosenfeld and Kak, 1982). Peak intensities in the original spectra were also tabulated after dividing their absorption values by that corresponding to the band at 1620 cm^{-1} (generally assigned to aromatic skeletal vibrations), obtaining relative optical densities in the IR range (wavelength cm^{-1}).

Finally, the in vitro respiratory activity of soils (soil C mineralization potential) was determined by estimating the CO_2 released from soil samples moistened to 60% of their water holding capacity at 24±1ºC, measured periodically for a period of 40 days with a Carmhograph-12 (Wösthoff) gas analyser (Almendros *et al*., 2005); the results referred to total mineralisation coefficient, taking into account that each soil had a different organic matter content, and was defined as milligrams of carbon released per 100 g of soil carbon per day.

Table 2. Analytical data of the Ah horizons of the soil studied

	SN (n=12)			SC (n=12)			CG (n=12)		
	Range Mean (SD)	Skew	Kurtosis	Range Mean (SD)	Skew	Kurtosis	Range Mean (SD)	Skew	Kurtosis
OC (g kg^{-1})	3.7-58.2 26.3 (15.42)	0.90	0.03	8.4-63.4 33.1 (16.82)	0.81	-0.08	1.0-30.5 17.8 (8.52)	-0.73	-0.15
N (g kg^{-1})	0.8-4.5 1.9 (1.08)	1.73	1.06	0.7-4.5 2.5 (1.11)	0.22	-0.21	0.3-2.6 1.5 (0.60)	-0.17	0.50
C/N	5-17 13 (3.12)	-1.89	3.36	10-20 13 (2.66)	1.73	2.80	3-15 11 (3.17)	-1.21	1.52
pH (H$_2$O)	4.9-7.4 6.3 (0.63)	-0.88	1.13	5.6-8.1 7.4 (0.73)	-1.50	1.53	5.2-8.6 7.7 (0.84)	-2.81	6.33
Ca^{2+}	0.75-18.20 6.24 (4.61)	1.69	2.64	9.0-55.5 31.69 (15.19)	-0.22	-0.91	6.75-45.75 31.56 (14.14)	-0.83	-0.43
Mg^{2+}	0.25-3.38 1.44 (0.88)	1.08	0.65	0.87-8.37 2.89 (2.19)	1.70	1.97	0.37-14.0 4.87 (3.87)	0.99	1.17
K$^+$	0.13-0.67 0.31 (0.16)	1.17	0.80	0.16-1.53 0.68 (0.42)	0.68	-0.29	0.16-1.86 0.91 (0.58)	0.58	-0.73
CEC	7.63-27.38 14.92 (5.72)	0.90	0.39	17.19-42.44 24.83 (6.63)	1.84	3.04	1.15-17.45 10.19 (4.22)	-0.71	0.86
Base Saturation (%)	7.1-83.7 53.9 (22.54)	-0.74	0.15	62.2-100 92.3 (13.75)	-1.75	1.27	75.3-100 97.9 (7.14)	-3.46	8.48
Free Iron (%)	2.25-4.04 3.09 (0.68)	-0.01	-1.38	0.84-4.91 1.99 (1.05)	2.10	4.04	0.15-3.52 1.26 (1.01)	0.96	0.57
Clay (%)	5.3-14.0 10.3 (3.04)	0.10	-0.43	12.5-51.9 24.9 (10.28)	1.60	2.84	4.4-34.9 13.0 (8.94)	1.30	1.88

Range (minimum-maximum), Mean value and SD (standard deviation). OC: total organic carbon; N: total nitrogen; Exchangeable bases (Ca^{2+}, Mg^{2+}, K$^+$) and cation exchange capacity (CEC) in cmol$_c$ kg^{-1}. SN: Sierra Nevada N.P.; SC: Sierra de Cazorla N.P.; CG: Cabo de Gata N.P.

2.3. Statistical analysis

The results were subjected to descriptive statistical analysis, analysis of variance (one-way ANOVA) to discriminate among the means by the Fisher´s least significant difference (LSD) procedure, principal components analysis (PCA), and correlations between variables with the Pearson´s correlation coefficient. Statgraphics Centurion XVI software was used (StatPoint Technologies, Inc.).

3. Results and Discussion

3.1. Basic soil characteristics

The characteristics of the topsoil samples analysed are shown in Table 2. In general, SN was characterised by a neutral to acid pH, coarse textures and low level of nutrients. SC was characterized by neutral to basic pH, a high level of nutrients and the exchange complex saturated with calcium. SC also had the highest organic carbon (probably related to a higher mean annual rainfall) and clay content, which would explain its higher cation exchange capacity. Finally, CG was characterized by a basic pH and a high level of nutrients.

As mentioned above, the results were grouped according to two basic criteria defined by the sampling zones, climatic and geochemical environments, and vegetation type. Thus it was possible to analyse and compare the influence of these factors on some properties related to SOM quality.

3.2. Influence of the sampling zone on soil organic matter quality

The selected characteristics of the SOM, grouped by sampling zone (i.e., lithology and climate conditions), are shown in Table 3. In organic matter fractionation, a significantly higher content in free organic matter (FOM) fraction was observed in SN than in SC or CG. The difference in biogeochemical conditions in the basic medium from the acid medium seems to be responsible for the significantly lower FOM fraction in SC and CG than in SN. The low condensation of humic substances can be observed in the prevalence of fulvic acids over humic acids (mean HA/FA ratios, related to the aromaticity and polymerization degree of humic substances, were less than one), mainly in samples from SN and SC. The humus fractions of total extractable humin (HUMIN) and non-extractable organic carbon (NEOC) are noteworthy. The HUMIN fraction had the highest means in CG and SC, significantly different from the SN samples. This fraction is made up of insolubilized humic colloids in a mineral matrix forming highly stable compounds (Stevenson and Cole, 1999; Almendros *el al*., 2005) that would greatly stabilize the soil. The NEOC fraction was significantly higher in SN (54.9%) than in SC where it was 44.2% or in CG where it was 43.3%. This larger fraction in SN, which is a medium with less biological activity, probably shows that it is a little-evolved fraction. According to Almendros et al. (1996), this fraction (called "insolubilisation humin") is usually made up of recalcitrant microbial and plant biopolymers, originally insoluble and strongly aliphatic, which is concentrated in the insoluble fraction of the SOM. It is not necessarily generated by long complex decomposition processes (López-Sangil and Rovira, 2013), and therefore, the results indicate a less mature humus in the acid environment of SN than in the other two areas.

Of the fundamental HA elemental composition ratios, the H/C ratio, which is related to aliphaticity, and the atomic O/C ratio, which is a direct reference to the degree of oxidation of the humic acid molecule and its carboxyl functional groups (Stevenson, 1994; Senesi *et al*., 2003), only the last ratio showed higher significant level of oxidation in samples from SC (0.64) and CG (0.63) than SN (0.56).

The E_4/E_6 ratio of soil HA decreases when the condensation and aromaticity of the humic substances rise and with increasing molecular weight (Stevenson, 1994; Fuentes *et al.*, 2006), which is typical of more mature, more evolved organic materials, and is therefore useful as a humification indicator. The E_4/E_6 ratio did not reveal significant differences in the three sample zones, although the group means were somewhat lower in CG and SC; are consistent with those commonly found in temperate areas (Oyonarte *et al*., 1994). The visible derivatographic spectroscopy of HA studied highlights valleys near 620 nm, 570 nm and 528 nm (D_{620}, D_{570} and D_{528}). These diagnostic spectral bands suggest the presence of perylenequinonic pigments derived from fungi (Oyonarte *et al*., 1994; Almendros *et al*., 2005). Table 3 shows the mean intensities of these bands in which differences may be observed, although not significant when grouping the samples by sampling zone, and which are generally higher in the basic mediums in SC and CG. In SN, some soil microbiological conditions are less favourable for these microbial metabolites. The constant presence of these substances might be considered characteristic of the humification processes in Mediterranean ecosystems (Sánchez-Marañón *et al*., 2002; Miralles *et al*., 2007; Miralles *et al*., 2012), playing a role in the accumulation of stable C forms in soil.

Assignment of the main infrared spectral bands in humic substances is primarily based on Haberhauer and Gerzabek (2001), Madari *et al.* (2006) and Tatzber *et al.* (2007). As shown in Table 3, the intensity of the 2920 cm^{-1} band implies significantly less presence of aliphatic C-H groups in CG in contrast with the clearly more aliphatic HA in SN. An intermediate mean was found in SC. With the advance of humification in the soil, humic substances become more aromatic and less aliphatic (Ding *et al.*, 2002).

Table 3. Selected parameters of the Soil Organic Matter in Mediterranean ecosystems soils (SE Spain). Grouping criterion: Sampling zone

	SN(a)	SC(b)	CG(c)	*P-value*	(*)
Sample size	12	12	12	-	-
Total organic carbon (OC), %	2.63	3.31	1.77	0.039	bc
Humus fractions, g C (100g soil C)$^{-1}$					
Free Organic Matter (FOM)	7.55	1.94	4.51	0.002	ab, ac
HA/FA ratio	0.8	0.7	1.0	0.218	-
Total extractable humin (HUMIN)	2.02	15.24	17.81	0.000	ab, ac
Non-extractable organic carbon (NEOC)	54.95	44.19	43.33	0.006	ab, ac
Elemental composition of HA					
Atomic H/C ratio	1.41	1.47	1.43	0.594	-
Atomic O/C ratio	0.56	0.64	0.63	0.003	ab, ac
Visible spectroscopy measurements in HA					
E_4/E_6 ratio	4.57	4.52	4.35	0.738	-
D_{620} (visible derivatographic spectroscopy, AU)	0.016	0.018	0.017	0.868	-
D_{570} (")	0.013	0.014	0.014	0.812	-
D_{528} (")	0.017	0.021	0.019	0.325	-
Infrared spectroscopy of HA (relative optical density of the main bands)					
2920 cm^{-1}	0.80	0.73	0.66	0.009	ac
1660 cm^{-1}	0.83	0.77	0.70	0.087	ac
1510 cm^{-1}	0.78	0.63	0.61	0.033	ac, ab
1460 cm^{-1}	0.89	0.74	0.70	0.012	ac, ab
1420 cm^{-1}	0.64	0.63	0.65	0.775	-
1380 cm^{-1}	0.65	0.63	0.65	0.827	-
1080 cm^{-1}	0.45	0.42	0.40	0.288	-
Soil respiratory activity Total mineralization coefficient (TMC), mg C (100g soil C)$^{-1}$ day^{-1}	1.36	1.19	1.33	0.790	-

SN: Sierra Nevada N.P. (acid metamorphic substratum); SC: Sierra de Cazorla N.P. (calcareous substratum); CG: Cabo de Gata N.P. (calcareous/volcanic substratum). AU: absorbance units. (*) Groups denote statistically significant differences at $p < 0.05$ level (LSD procedure).

These processes result in the concentration of condensed aromatic structures and the elimination of lateral chains, and macromolecules become more stable and increasingly more resistant to biodegradation (Stevenson, 1994). Aliphatic and N-containing groups are assigned to the 1660 cm^{-1} band. High intensities in this area of the spectra indicate high content in little-evolved forms of nitrogen, mainly characterized by higher protein content. In this band, optical density is significantly lower in CG, highest in SN and intermediate in SC. The less-stabilized HA fraction showed a similar trend according to the band at 1510 cm^{-1}, where CG intensity was the lowest, followed by SC, and SN was the highest. This again suggests that SN is the least favourable environment for humus evolution complexity. The presence of lignin can usually be detected by characteristic spectroscopic patterns at around 1460, 1420 and 1380 cm^{-1} (Almendros *et al.*, 2003). Only the intensity at 1460 cm^{-1} (aliphatic C-H groups) is sufficiently statistically significant to separate CG and SC, which have similar group means, from SN samples, which have much higher constituent

lignin. Lignin decomposes slowly and is a recalcitrant fraction in soil (Sleutel *et al*., 2007). Thus, although the large amount present suggests less evolved humus, from the point of view of its persistence in soils, selective enrichment of recalcitrant molecule classes contained in plants, like lignin or phenols, is of interest. SN samples also showed higher intensities for the band at 1080 cm^{-1}, typical of C-O and C-H deformations, which although not statistically significant, indicates the presence of polysaccharides or carbohydrate-like substances, reflecting the influence of recent plant material.

The in vitro respiratory activity, expressed as total mineralisation coefficients (TMC), provides an estimate of the intrinsic biodegradability of organic matter in soil and its degree of maturity and stability; being considered a key factor of humus quality in terms of soil C sequestration (Almendros *et al*., 2005). The organic matter TMC was very similar to what is commonly reported in temperate areas (Oyonarte *et al*., 1994). These results are somewhat, though not significantly, different from the groups studied, with a higher mean for SN, where therefore, the organic matter accumulated is more biodegradable, i.e., more readily available to microorganisms. Also, there is a significant negative relationship between soil respiratory activity (TMC) and HA content (r = -0.517; $p < 0.01$), which shows that this organic matter fraction is more intrinsically chemically recalcitrant, and its increase indicates stabilization of SOM.

The typical Mediterranean climate also characterizes mountain areas with cold winters and very dry summers. In SN, the lower mean annual temperature and higher altitude (Table 1), combined with the acidity of the schistose substratum and low base saturation of the soils, might explain the differences in the progress of humification, with a higher resistance of their organic C to be transformed into more evolved forms by microbial activity. As suggested by Zeraatpishe and Khormali (2012), climate and parent material are the main factors responsible for SOM accumulation and decomposition. On the contrary, and coinciding with a previous study by Miralles *et al*. (2007), strong seasonal moisture changes

typical of the continental Mediterranean climate, associated with high solar irradiation, especially intense in CG, could be favouring the formation and maturation of HA observed in the latter. Also, and according to Djukic *et al*. (2010), higher pH (as in CG and SC, Table 2) implies favourable conditions for decomposition and may lead to higher humification and accumulation of aromatic structures and HA stability.

3.3. Influence of the sampling zone and vegetation type on soil organic matter quality

Table 4 shows the general characteristics of SOM taking into account the lithology and climate conditions along with vegetation type. The humus fractions with statistical significance between the groups are FOM, HUMIN and NEOC. The highest average FOM fraction content (11.7%) is observed in the samples beneath *Pinus* species reforestation in an acid medium (SNR), the biogeochemical conditions of which would favour a higher content in plant residues. The HUMIN fraction enables the samples from SC and CG to be differentiated from SN, which with clearly lower averages, would have less capacity to form stable organic-mineral complexes. The NEOC fraction is the main fraction in the samples beneath reforestations with *Pinus* both in basic and in acid environments (SCR and SNR) as well as beneath forest with *Quercus* in an acid environment (SNB). Therefore, those samples taken from soils subjected to reforestation are clearly separated by their lower humification.

The atomic O/C ratio, from the elemental composition of the HA and the only one that is statistically significant, suggests that the highest carboxylation in the HA comes from the SC and CG sites regardless of the type of vegetation.

It may be concluded that scrubland, regardless of which area it is in (SNM, SCM and CGM), has lower E_4/E_6 ratios, though not statistically significant, whilst reforested vegetation tends to have a higher ratio (SNR and SCR), and therefore, a tendency to less macromolecular complexity in the HA.

Table 4. Selected parameters of the Soil Organic Matter in Mediterranean ecosystems soils (SE Spain). Grouping criterion: Sampling zone and vegetation type

	SNB(a)	SNR(b)	SNM(c)	SCB(d)	SCR(e)	SCM(f)	CGM(g)	*P-value*	(*)
Sample size	4	4	4	4	4	4	12	-	-
Total organic carbon (OC), %	3.75	1.78	2.36	4.15	3.07	2.70	1.77	0.047	dg, ag, bd, ab
Humus fractions, g C $(100g \text{ soil C})^{-1}$									
Free Organic Matter (FOM)	6.45	11.71	4.45	2.20	1.21	2.40	4.51	0.000	bg, bd, bf, ae, be, ab, bc
HA/FA ratio	0.8	0.7	0.8	0.6	0.6	0.7	1.0	0.760	-
Total extractable humin (HUMIN)	1.91	2.10	2.05	14.87	14.52	16.32	17.81	0.000	ag, cg, bg, ad, cd, bd, af, cf, bf, ae, ce, be
Non-extractable organic carbon (NEOC)	59.28	56.68	48.88	38.24	52.68	41.66	43.33	0.006	ag, bg, de, ad, bd, af, bf
Elemental composition of HA									
Atomic H/C ratio	1.41	1.40	1.43	1.46	1.45	1.50	1.43	0.972	-
Atomic O/C ratio	0.58	0.56	0.54	0.63	0.65	0.63	0.63	0.059	cg, bg, cd, cf, ce, be
Visible spectroscopy measurements in HA									
E_4/E_6 ratio	4.60	4.87	4.24	4.71	4.83	4.25	4.35	0.748	-
D_{620} (visible derivatographic spectroscopy, AU)	0.014	0.012	0.022	0.016	0.015	0.021	0.017	0.149	bf, bc
D_{570} (")	0.011	0.010	0.017	0.013	0.012	0.016	0.014	0.119	bf, ac, bc
D_{528} (")	0.014	0.013	0.022	0.019	0.016	0.029	0.019	0.037	fg, df, ef, af, bf
Infrared spectroscopy of HA (relative optical density of the main bands)									
2920 cm^{-1}	0.83	0.81	0.76	0.76	0.70	0.74	0.66	0.104	ag, bg
1660 cm^{-1}	0.86	0.91	0.72	0.80	0.69	0.80	0.70	0.119	bg, be
1510 cm^{-1}	0.78	0.97	0.60	0.65	0.59	0.67	0.61	0.007	bg, bd, bf, be, bc
1460 cm^{-1}	0.89	1.02	0.76	0.76	0.73	0.72	0.70	0.027	ag, bg, bd, bf, be, bc
1420 cm^{-1}	0.66	0.69	0.65	0.67	0.67	0.68	0.67	0.833	-
1380 cm^{-1}	0.64	0.70	0.60	0.64	0.63	0.63	0.65	0.521	bc
1080 cm^{-1}	0.44	0.51	0.39	0.43	0.39	0.44	0.40	0.163	bg, be, bc
Soil respiratory activity									
Total mineralization coefficient (TMC), mg C $(100g \text{ soil C})^{-1} \text{ day}^{-1}$	1.32	1.86	0.89	1.16	1.43	0.98	1.33	0.384	bc

SN: Sierra Nevada N.P. (acid metamorphic substratum); SC: Sierra de Cazorla N.P. (calcareous substratum); CG: Cabo de Gata N.P. (calcareous/volcanic substratum); B: natural forests; R: pine reforestations; M: scrublands. AU: absorbance units. (*) Groups denote statistically significant differences at *p* < 0.05 level (LSD procedure).

Statistically significant differences were observed in the intensities of D_{620}, D_{570} and D_{528} with derivatographic spectroscopy, indicating, as mentioned above, the presence of perylenequinonic pigments derived from fungi. There are more presence of these substances in the samples from scrubland vegetation, mainly in SNM and SCM. Reforestation in the acid environment (SNR) does not appear to favour the development of the microbial populations responsible for the concentration of quinoid pigments.

Some interesting differences amongst the groups were highlighted by IR spectroscopy of the HA. The contribution of aliphatic C-H vibrations (2920 cm^{-1} band) to the structure of HA is larger in the reforested (SNR) and Quercus forest (SNB) areas, and lower in the reforested areas in SC, and even lower in the

scrubland in CG, confirming the pattern of less advanced humification in the acid environment of Sierra Nevada. Significantly higher intensities of the bands at 1660 cm^{-1}, 1510 cm^{-1} and 1080 cm^{-1}, are observed in the SNR samples, the group with organic matter with the least transformation. A clear and significant difference is found in the samples from beneath reforested pine trees in an acid medium, in which the high intensities of the peaks at 1460 cm^{-1} and 1380 cm^{-1} (accumulation of lignin in the HA) are an indicator of less evolved humic substances. The intensity of these bands also shows a clear reduction in basic medium. Figure 2 shows the representative resolution-enhanced IR spectra of a HA sample from SNR (a), coinciding with the highest E_4/E_6 ratio, and from CGM (b), coinciding with the lowest E_4/E_6 ratio.
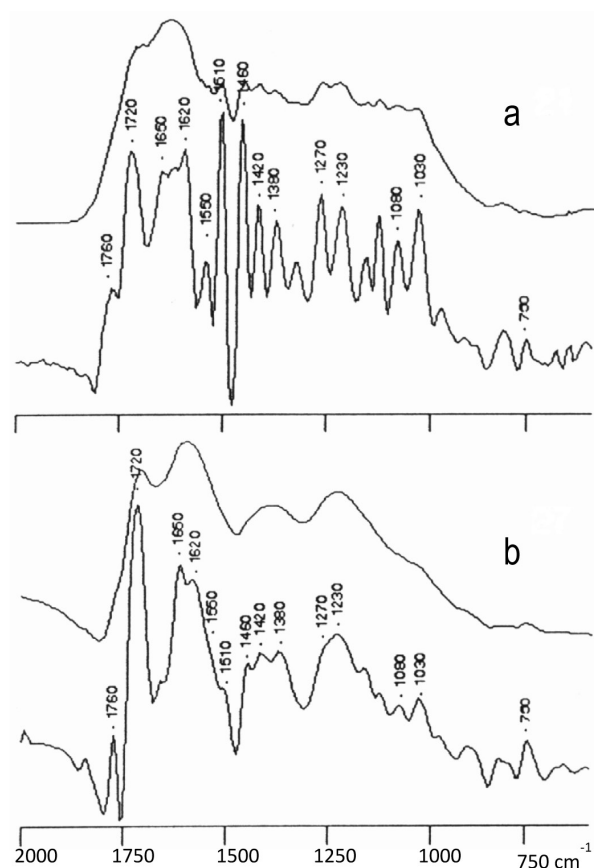
Figure 2. FTIR spectra and resolution-enhanced IR spectra (bottom) of the 2000-600 cm^{-1} region, representative of humic acids from SNR (a) and CGM (b) ecosystems soils.

The average TMC is higher in the SNR group (1.86 mg C (100 g soil C)$^{-1}$ day^{-1}), hence the samples beneath Pinus in an acid medium are less stable and their organic material tends to be more biodegradable.

In general, the HA sampled from the soils beneath *Quercus* are more evolved than under *Pinus*, with less humification observed overall beneath the latter. Results would seem to indicate that the areas reforested with *Pinus* species have a more negative impact on the acid SN environment than on the basic SC environment. In the latter, vegetal remains from pine trees have undergone a deeper transformation and more efficient humification by the soil environment. In this sense, a large percentage of the area reforested with pine trees in SC may have been more efficiently stabilised than in SN, thereby achieving a certain balance with the environmental conditions due to more favourable geochemical conditions and biological activity. A biogenic background representative of the original *Quercus* vegetation could be overlapping the organic compounds derived from *Pinus* reforestations and would explain the scant analytical differences

between samples beneath natural forests (SCB) and beneath reforested areas (SCR). All the scrublands, regardless of the edaphoclimatic zone they belonged to, were observed to tend toward generation of more highly evolved humus. Moreover, the scrubland on the basic substratum in the Cabo de Gata N.P. (CGM), showed a more favourable tendency in the evolution of the organic fraction, possibly affected by the mild winter temperatures, the effect of hidden rain, and to the good adaptation of the scrubland to the ecological conditions present in the area.

In the samples from the basic substratum, a tendency to formation of a somewhat more evolved type of humus was observed. This may be due to the greater biological activity associated with the type of vegetation (including pines in SC) and a thicker and more varied vegetation which intensifies the biogeochemical cycle by supplying more easily decomposable remains, and to the type of lithological substratum that releases nutrients into the medium favouring biological activity of both micro-organisms and plants.

Table 5. Selected parameters of the Soil Organic Matter in Mediterranean ecosystems soils (SE Spain). Grouping criterion: vegetation type

	B(a)	**R**(b)	**M**(c)	*P-value*	(*)
Sample size	8	8	20	-	-
Total organic carbon (OC), %	3.95	2.43	2.08	0.008	ab, ac
Humus fractions, g C (100g soil C)$^{-1}$					
Free Organic Matter (FOM)	4.34	6.46	4.08	0.376	-
HA/FA ratio	0.7	0.6	0.9	0.344	-
Total extractable humin (HUMIN)	8.39	8.31	14.36	0.118	-
Non-extractable organic carbon (NEOC)	48.76	54.68	44.11	0.044	bc
Elemental composition of HA					
Atomic H/C ratio	1.43	1.43	1.45	0.948	-
Atomic O/C ratio	0.60	0.61	0.61	0.916	-
Visible spectroscopy measurements in HA					
E_4/E_6 ratio	4.53	4.85	4.31	0.173	-
D_{620} (visible derivatographic spectroscopy, AU)	0.015	0.013	0.019	0.081	bc
D_{570} (")	0.012	0.011	0.015	0.032	bc
D_{528} (")	0.017	0.014	0.022	0.030	bc
Infrared spectroscopy of HA (relative optical density of the main bands)					
2920 cm^{-1}	0.80	0.76	0.69	0.093	ac
1660 cm^{-1}	0.83	0.81	0.73	0.166	-
1510 cm^{-1}	0.71	0.78	0.62	0.085	bc
1460 cm^{-1}	0.83	0.88	0.71	0.046	bc
1420 cm^{-1}	0.64	0.66	0.63	0.580	-
1380 cm^{-1}	0.64	0.67	0.63	0.485	-
1080 cm^{-1}	0.44	0.45	0.41	0.335	-
Soil respiratory activity Total mineralization coefficient (TMC), mg C (100g soil C)$^{-1}$ day^{-1}	1.24	1.65	1.17	0.178	-

B: natural forests; R: pine reforestations; M: scrublands. AU: absorbance units. (*) Groups denote statistically significant differences at *p* < 0.05 level (LSD procedure).

3.4. Influence of the vegetation type on soil organic matter quality

When the characteristics of the SOM of the samples were compared by vegetation type (Table 5) no great differences among the groups were observed. Nonetheless, some differences are worthy of mention. The NEOC fraction of the humus turns out to be significantly higher in the samples from beneath pine trees (R), which would confirm their lesser evolution. Furthermore, although not statistically significant, the samples beneath scrubland (M) have a larger proportion of carbon in the HUMIN fraction, higher HA/FA ratio and lower proportion of FOM than in the other two plant formations.

The visible spectra reveal less condensation and lower molecular weight of the humic substances underneath reforestations (R), shown by their highest extinction E_4/E_6 ratio. The concentration of fungal pigments in HA is related to the type of vegetation, and turns out to be significantly higher both in scrubland (M), where content reaches a maximum, and in natural forest (B), both plant formations in which the microbial synthesis mechanisms responsible for the accumulation of perylenequinonic pigments are intensified in the humus formation. Amongst the main IR spectra bands related to the presence of lignin, the 1460 cm^{-1} band for the samples from beneath pines (R) is especially significant. Higher level of aliphaticity (2920 cm^{-1}) and a less stabilized HA fraction (1510 cm^{-1}) were also found in the samples beneath *Quercus* (B) and *Pinus* (R). Hence, several of the characteristics analysed indicate that the organic remains of soils beneath *Quercus* were more decomposed than those from coniferous forests, which would enrich the humic substances in recalcitrant organic components giving rise to limited decomposition.

The results give an idea of more favourable humification progress beneath scrubland vegetation (M) as confirmed by some variables such as FOM, HA/FA, HUMIN, 1660 cm^{-1} and 1080 cm^{-1} IR spectra, and TMC, although not statistically significant. The wide plant diversity in the scrubland, could affect below-ground diversity and greater microhabitat heterogeneity, leading to an increase in decomposition rates and evolution of humus. This implies that the scrubland might provide greater stability for the surface horizons of the soils, and therefore, for the related ecosystems, than the other plant formations studied.

## 3.5. Potential of spectroscopic data for assessing variation in humus

The results show the potential of spectroscopic data for evaluating the mechanisms of humification that lead to maturation of HA. Characterisation of the extent of humification of humic substances could also be of special interest, because, according to Santos *et al.* (2010), more humified substances are generally more chemically stable, increasing the residence time of organic matter in the environment and consequently improving soil quality.

The optical density of HA solutions adjusted to a constant concentration is frequently taken as the most valuable surrogate indicator of aromaticity and maturation of humic matter (Almendros *et al.*, 2003). Following this criterion, and analysing the main HA spectroscopic data, a significant correlation is observed in which the higher the E_4 optical density, the higher the fungal pigment content (Figure 3a), mainly with respect to the intensity at 528 nm in the second derivative spectra. This relationship means that the increase in aromaticity and condensation with humification in the samples is a product of intensified biogeochemical activity with accumulation of microbially reworked HA and microbial metabolites.

When the IR band at 1460 cm^{-1} (the most significant in the lignin pattern) is correlated with the E_4 optical density (Figure 3b), or the E_4/E_6 ratio (Figure 3c), a significant negative correlation is observed in the first case, and as expected, positive in the second. Hence, in the sites under study, a clear trend towards a less dark color is observed in those HA with better defined

lignin pattern. The tendency of less humification with accumulation of lignin is validated by a positive correlation between the IR band at 1460 cm^{-1}, and among others alkyl compounds, with the protein IR band at 1660 cm^{-1} (Figure 3d). These results show a decrease in humification in samples where accumulation of plant macromolecules, such as lignin structures, predominates, and is the major humification mechanism.The representation in space defined by the first two factors found with principal component analysis (Figure 4) showed two clusters based on quality descriptors interpreted as different humification mechanisms.

The right-hand cluster showed descriptors related to oxidation and microbial quinoid content in the HA, and degree of humification (HA/FA ratio), i.e., with high biogeochemical organic matter transformation and more advanced and complex stages of humification. The left-hand cluster compiled all descriptors related with high organic carbon (OC, NEOC and FOM) and raw humus associated with slightly altered macromolecules in the HA fraction (higher aliphaticity, strong lignin patterns, and higher polysaccharides and protein contents). Less stability of the organic matter expressed as TMC would have to be stressed in this cluster.
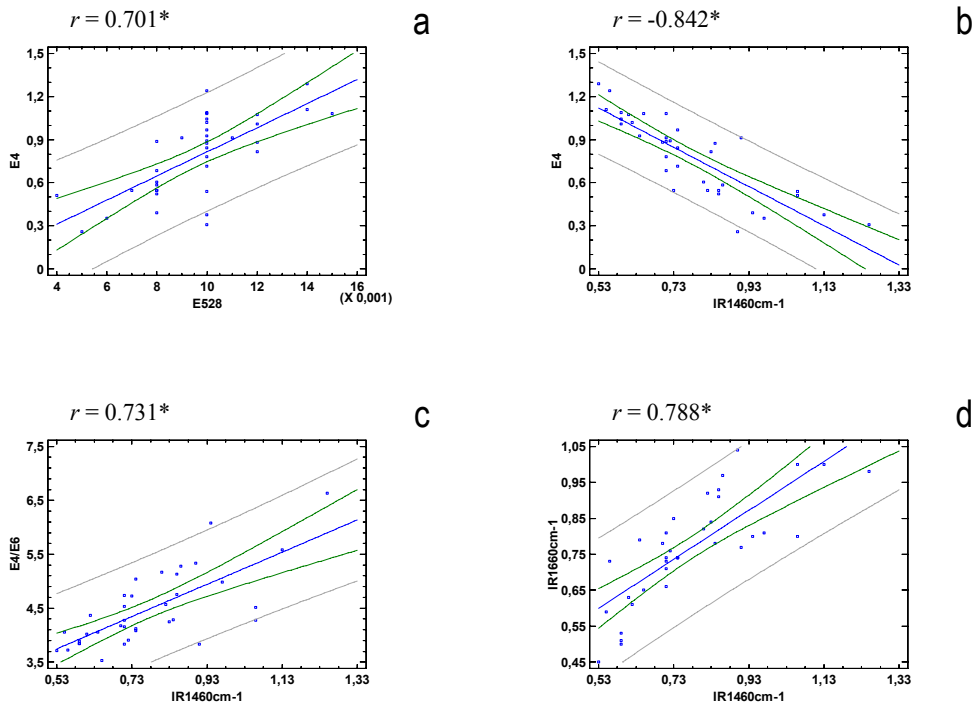
Figure 3. Correlations between the main spectroscopic parameters to establish the origin of humus variation. *Statistical significance level: *p-value* < 0.001; n = 36.
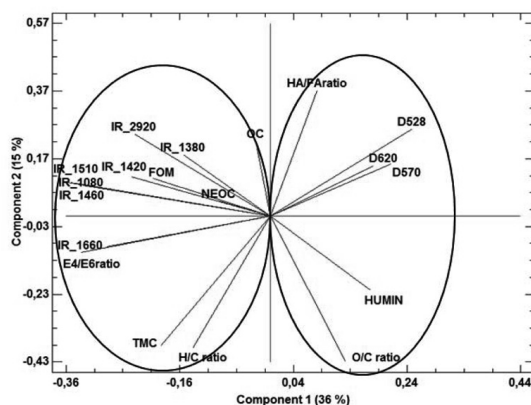
Figure 4. 2D scatter plot of PCA and variance explained (%) by each component of soil organic matter quality parameters. Parameter abbreviations from Tables 3, 4 and 5.

4. Conclusions

The set of analyses used to characterize the chemical composition and quality of the organic matter in the environments studied suggests uniform results of humification, probably due to the Mediterranean climate, which has strong seasonal changes that act on Mediterranean vegetation in a unique way that is well adapted to the different parent materials (calcareous, acid metamorphic and calcareous/ volcanic rocks). Differences among the sampling zones indicate that the acid environment in Sierra Nevada, with an acid schistose substratum and harsh climate conditions, can be considered the least favourable for a higher complexity in humus evolution. In this acid environment in Sierra Nevada, the species of *Pinus* showed lower efficiency in the humification mechanisms, clearly explained by the physicochemical nature of the humic substances. Moreover, in the basic environment in Sierra de Cazorla, the differences between the samples beneath natural forest and reforested areas are scant, as a result of some more favourable geochemical conditions and biological activity, as compared to the acid environment in Sierra Nevada.

The analysis of the samples beneath the three types of vegetation reveal a certain differentiation in the characteristics of the humus according to the nature of the organic remains, much less obvious differences than would be expected bearing in mind the different characteristics of the plant remains and vegetation types, interpreted as a certain resilience of the humus formations in the sites under study. Beneath *Pinus* vegetation, the humus proves to be less evolved and stable than in natural forests and scrubland. Results would seem to indicate that the scrubland is capable of creating humus with more favourable characteristics for greater stability in the surface horizons of the soils studied.

These results provide valuable information on sustainability in vegetation management practices considering lithology and climate conditions in areas with high risk of degradation, such as Mediterranean environments.

Acknowledgments

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