WATER DISSOLVED ORGANIC MATTER UNDER WOODY VEGETATION PATCHES IN SEMI-ARID MEDITERRANEAN SOILS. UV-VIS AND HPLC CHARACTERIZATION.

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Abstract

The impact of woody vegetation patches on soil dissolved organic matter (DOM) in a semi-arid steppe was studied. DOM from forty-two patch and inter-patch soil samples, from four different sites in the Southeast of Spain, was extracted with water and analyzed by UV-Vis spectrophotometry. Several UV-Vis indexes, related to chemical

characteristics of soil organic matter, were calculated and the occurrence of low molecular weight organic acids determined by HPLC with UV-Vis detector. Three sources of statistical variation were found: soil location (patch or inter-patch), site, and in to a lesser extent the dominant plant species growing in the patch. Patch soils were richer in DOM and organic acids than inter-patch soils. The effect of site, was related to climatic conditions, as average annual rainfall and temperature. Patch soils act as sources of DOM that may be leached to inter-patch soil. Soluble organic molecules may increase soil fertility under woody patches and favor the establishment of other plants. Fumaric acid was present in all soils, together with other organic acids with aliphatic chains and aromatic rings. Dominant species affected organic matter accumulation, but their impact on SOM quality was unclear. Thus, patches dominated by *Ephedra fragilis* accumulated higher quantities of DOM than those dominated by other species, whilst *Rhamnus lycioides* patches showed the highest accumulation of fumaric acid.

Keywords: arid soils, DOM, woody patches, HPLC, UV-Vis

1. Introduction

Soil organic matter (SOM) is a complex mixture of organic molecules with different structural and functional characteristics. Size, composition and structure of the organic components may vary greatly depending on the origin and the age of the sample (Chen et al. 2002).

Soils that are rich in humified organic matter are more stable and resistant to degradation. The quality of soil organic matter can be more important for soil fertility and dynamics than its quantity, especially in drylands, where the risk of soil degradation and desertification is very high (Aranda and Comino, 2014).

In arid and semi-arid drylands, woody vegetation often forms discrete patches amid a matrix of herbaceous vegetation and bare soil (Aguilar and Sala, 1999; Ludwig and Tongway, 1995). Patch-forming species frequently act as keystone species, as they strongly affect community composition and ecosystem functioning (López and Moro, 1997; Maestre and Cortina, 2004; Maestre et al., 2009). Woody vegetation patches form fertility islands in areas with severe limitations of water and nutrients. Patch soils are commonly richer in organic matter than inter-patch soils. This improves soil physicochemical properties, and may contribute to reduce soil erosion and further degradation (Vasquez-Mendez et al. 2010). Improved soil fertility under woody patches may also facilitate the recruitment of other plant species, increasing patch area and expanding its influence (Castro et al., 2004; Amat et al., 2014). However, despite the key role of woody patches in drylands, and the accumulation of SOM underneath them, our knowledge on its composition and properties is still scarce.

The most active and mobile fraction of SOM is dissolved organic matter (DOM; Corvasce et al., 2006). DOM can be obtained by extracting with different aqueous solutions (Karavanova, 2013; Zsolnay, 2003;). Dissolved organic matter and water extractable organic matter (WEOM) consist in complex mixtures of organic compounds. Their composition is similar to that of SOM as a result of the equilibrium between liquid and solid phases in soils (Zsolnay, 1996). DOM influences soil fertility, through mineral weathering, metal transport and biological activity (Chantigny, 2003).

DOM is usually characterized using spectroscopic methods. SOM has a strong absorbance in the UV-Vis range, due to the presence of aromatic groups and other organic moieties (Gu et al., 1995; Traina et al., 1990). Usually the absorptivity is higher

in the UV region, because of the $\pi \rightarrow \pi^*$ of benzenes and decreases as the wavelength increases (Chen et al. 2002; Traina et al., 1990).

The absorptivity in the visible region is attributed to the condensation of aromatic rings and organic molecules of high molecular weight (Chen et al., 2002). Although the UV-Vis spectra of soil organic matter, present few defined features, the relationships in absorbance at specific wavelengths and other indices, have been linked to chemical properties of these compounds, and the ratio E2/E3 is inversely correlated with the molecular weight and the amount of aromatic compounds (Minor et al. 2014; Peuravuori and Pihlaja, 1997). The relationship E4/E6 is inversely correlated to the condensation of aromatic rings (Minor et al. 2014; Purmalis and Klavins, 2014). Using size fractionation of DOM by ultrafiltration and gel filtration chromatography, Helms et al. (2008) found that the relationship of the slope of the spectrum in the 275-295 nm and 350-400 nm regions (SR index), was related to the molecular weight of the organic molecules. Specific absorbance at 254 nm and 280 nm ($SUVA_{254}$, $SUVA_{280}$) are directly related to the content of aromatic structures in humic substances (Peuravuori and Pihlaja, 1997, Weishaar et al., 2003). $SUVA_{280}$ is also related to the molecular weight, the aromaticity and the degree of humification of DOM. The later is commonly correlated with $\Delta \log k$, (Cunha et al. 2009, Purmalis and Klavins, 2013). Finally, the coefficient E270/400 (the ratio of absorbances at 270 and 400 nm) represents the balance between phenolic/quinoid moieties and carboxylic aromatic molecules (Purmalis and Klavins, 2013; Uyguner and Bekbolet, 2005).

Organic acids are an active part of DOM, and represent between 1-3% of its mass (van Hees et al., 1999). Organic acids affect soil pH and enzymatic activity, and play a key role in nutrient uptake, soil formation and ecological interactions (Vranova et al., 2013).

Organic acids are particularly important in calcareous soils where Ca excess may alter metabolic processes, and must be neutralized by precipitation (López-Bucio et al., 2000). Soil organic acids can be easily identified by HPLC (Ding et al. 2006; van Hees et al., 1999). Many HPLC systems are equipped with UV-Vis detectors, so the spectra obtained from individual peaks can be compared to the data obtained from UV-Vis spectra of DOC samples, in order to better understand DOM properties.

The objective of this paper is to characterize DOM under woody vegetation patches and in open areas in a semi-arid steppe, and explore DOM properties affecting soil fertility and ecological interactions. Semi-arid steppes are particularly suitable to develop this type of studies because (i) vegetation cover is discontinuous and there is a strong interaction between vascular plants and the soil underneath them, (ii) woody patches are key components of these steppes affecting ecosystem and landscape dynamics, and (iii) semi-arid steppes cover large areas and are particularly prone to desertification.

2. Materials and methods

The soil underneath forty-two woody patches was sampled together with the corresponding 42 inter-patch areas in four *Stipa tenacissima* L. steppes of Alicante (Aigües, Campello, Orihuela and Tibi), in southeast Spain (from 678032 E, 4242106 N to 731450 E, 4267575 N). Patches are mono and pluri-specific assemblages of resprouting shrubs as *Pistacia lentiscus* L., *Quercus coccifera* L., *Ephedra fragilis* Desf., *Juniperus oxycedrus* L. and *Rhamnus lycioides* L. (Amat, 2015; Maestre and Cortina, 2005;). Interpatch soil samples were taken at a minimum distance of 2 m from the edge of any patch to avoid patch influence. Four subsamples from each patch or interpatch area were taken and mixed in a plastic bag for subsequent analysis. Soils are

classified as Calciorthids and Torriorthents (Soil Taxonomy, 1994). Bedrock is calcite in Aigües, Campello and Tibi, and dolomite mixed with calcite in Orihuela. Site was also characterized by climatic data (annual and monthly average rainfall, and annual and monthly average, maximum and minimum temperature), altitude, location (UTMx and UTM_y coordinates). Patches were characterized in terms of their surface area, maximum height, aspect and the composition of patch-forming large resprouting shrubs (Amat et al., 2015).

Total organic carbon (TOC) was measured as oxidable C, and total N content by semi-micro-Kjeldahl distillation (Tecator Kjeltex Auto 1030 analyzer, Hogana, Sweden). Dissolved organic carbon (DOC) was obtained by shaking 10 g of soil with deionized water (100 mL), centrifugation at 4,000 rpm for 10 min, and filtration through 0.45 µm cellulose nitrate filters (Gigliotti et al., 2002). Fifty milliliters of the solution containing DOC were freeze-dried for elemental analysis. The remaining was used for UV-Vis analysis. Elemental analysis was conducted on a CHNS elemental microanalyzer with Micro detection system TruSpec LECO. UV-Vis analysis was registered between 200 and 800 nm in a 1 cm path-length quartz cell (Jasco UV-Vis spectrophotometer). Several absorbance relationships were calculated: E₂/E₃ (ratio between the absorption at 250 nm and absorption at 365 nm), E₄/E₆ (ratio between the absorption at 465 nm and absorption at 665 nm), the slope of the spectrum in the 275-295 nm and 350-400 nm regions (SR index), SUVA₂₅₄, SUVA₂₈₀ (the absorbance at 254 nm or 280nm divided by the concentration of C in the sample in mg.L⁻¹). The quotients E₂₈₀/E₄₇₂, E₂₈₀/E₆₆₄, E₄₇₂/E₆₆₄ (i.e. the quotients between the absorption at 280, 472, 664) represent molecular relationships between low (E₂₈₀); medium (E₄₇₂) and high (E₆₆₄) degree of humification, (Purmalis and Klavins, 2013). Finally, Δlogk defined as the

difference between the logarithms of the absorbances at 400 and 600nm. Organic acids were determined in 1:4 (w/v) soil/water extracts. Soil water suspensions were stirred for 4h at 20°C and centrifuged at 15000g for 5 min according to Mimmo et al. (2008). The supernatant was collected and frozen at –20°C until further analysis. Low molecular weight organic acids were separated by reversed-phase high performance liquid chromatography (HPLC) using a C18 column at 30°C, in isocratic elution with NaH₂PO₄ (20 mM) as carrier solution at a flow rate of 0.5 mL min⁻¹. Organic acids were detected at 210 nm using a photodiode array UV-VIS detector model SPD-M6A. Standard acids were prepared as individual stock solutions, using SigmaTM free acids, and then combined to give diluted reference standards. Organic acids were identified by comparing retention times of unknowns to pure organic acids, UV-Vis spectra and by standard additions. Although soil pH was always above 7, and organic acids were in their deprotonated form, we will refer to them as “organic acids” for simplicity. We performed an exploratory analysis of SOM properties by using Principal Component Analysis–We used one-way analysis of variance for a fixed factor with 4 levels to study the effect of site on organic matter characteristics (TOC, DOC and organic acids content) for interpatch soils. Finally, we used correlation analysis to relate OM properties and climatic variables. Statistical analyses were performed using the SPSSTM and UnscrambleTM software (SPSS for Windows, Version 21.0SPSS Inc, Chicago, IL; UNSCRAMBLE (Camo Software)

3. Results

3.1. Exploratory analysis

The principal component analysis reduces the number of dimensions in samples described by a high number of variables (Hassouna et al., 2010). The first component of the PC (PC1) explained 40% of the variation in SOM properties and was related to the

amount of soil organic C, and SUVA₂₅₄ and SUVA₂₈₀ (Fig. 1). It was thus related to the C content and the content of aromatic structures. Axis PC2 explained 24% of the variation and was related to molecular size and the degree of humification (E2/E3, E4/E6, E472/E664, E280/E664). Finally, PC3 explained 12% of variation and included similar variables as PC2 (data not shown)

Principal component analysis clearly separated patch and interpatch soils along axis PC1 (Fig. 1). This was the main difference observed, owed to higher TOC and DOC content in patch respect to interpatch soils. Soils from each study area were also grouped, suggesting that site had a significant influence on patch and interpatch soil characteristics. Aigües soils had positive values of PC2, whereas other soils showed negative values along this axis, and variable values of PC1. Finally, the only patches dominated by *Ephedra* were isolated, as they showed relatively high values of PC1 and PC2.

3.2. Patch effect on soil organic matter

3.2.1. Organic carbon content

TOC values ranged between 0.8% and 13%, most soils being below 6%. TOC content of patch soils almost doubled TOC content of interpatch soils (Table 1). Higher levels of organic matter in patch soils respect to interpatch soils have been frequently reported in drylands (Cortina and Maestre, 2005; Vasquez-Mendez et al., 2010). The amount of DOC ranged between 0.04 and 0.4 (C mg soil g⁻¹), corresponding to 0.08-1.8 DOC (mg TOC g⁻¹). As a result of the large variability in TOC and DOC content, the amount of C stored as DOC in the first 5 cm soil ranged from 46 to 460 kg ha⁻¹. DOC values were also higher for patch soil than for interpatch soil (Table 1). In contrast, the ratio DOC/TOC did not differ between patches and inter-patches (Table 1).

Table 1. Mean values of C content in patch and interpatch soils. Different letters indicate statistically significant differences between soil types ($p < 0.001$)

	patch	interpatch
TOC (C mg.soil g ⁻¹)	56a	28b
DOC (C mg.soil g ⁻¹)	0.18a	0.11b
DOC/TOC (mg.g ⁻¹)	0.40a	0.52a

There was a positive relationship between TOC, and particularly DOC, measured in patch and interpatch soils (Fig. 2). This may reflect similarities in C dynamics at the microsite scale that persist despite the influence of woody patches. In addition, despite that the litter layer is commonly confined to the limits of the projected canopy (Amat, 2015), and patch effect on the establishment of other plants is spatially limited (Amat et al., 2015), roots of patch-forming shrubs may extend beyond their canopy, contributing to OM accumulation in inter-patch areas.

DOC decreased as mean annual rainfall increased (Fig. 3). Although the decrease occurred both, in patch and in interpatch soils, the slope was slightly higher in patch soils ($-7.0 \pm 3 \times 10^{-5}$ and $-4.8 \pm 2 \times 10^{-5}$, respectively), suggesting a possible leaching of DOC from soil patches. Tipping al. (1999), studying three soil types with contrasted organic C levels, found that DOC was mainly leached from the soil with higher organic matter content.

3.2.2. Sulfur, carbon and nitrogen concentration of DOM

Elemental analysis showed no traces of S in DOM. The average values of the H/C ratio in patch and interpatch soils were similar: 1.5 and 1.4, respectively. Humic substances with high aromaticity commonly show H/C ratios close to 1 (Kang et al. 2002). He et al. (2014) calculated H/C ratios for compost derived DOM between 1.8 and 2, which revealed the prevalence of aliphatic groups. The intermediate value obtained in our study suggests the balanced presence of both types of organic molecules, and it is similar to those found by Aranda and Comino (2014) in reforested soils in southern Spain. Aranda and Comino (2014) study included oak and pine forests, and scrublands with *S. tenacissima*.

The C/N ratio of SOM and DOM differed widely (17.5 vs. 26.5 respectively). Haney et al. (2012) found that the C/N ratio of DOM better represented microbial activity than the C/N ratio of the total organic matter; thus, in soils where C/N values are above 20, most N may be immobilized by microorganisms and no net N mineralization may be expected. In contrast, no differences were observed in the C/N ratio of SOM and DOM between patch and interpatch soils, despite substantial differences in TOC and DOM content. Organic matter inputs as litterfall and fine root mortality are probably higher underneath woody patches, and fresh organic residues commonly show high C/N ratios (Vallejo, 1993; Aerts, 1997). However, soils show a strong capacity to homogenize SOM properties (decay filter or chemical convergence hypothesis, Melillo et al., 1989; Wickings et al., 2012), which may explain the absence of differences in the C/N ratio between patches and interpatches.

3.2.3. UV-Vis indices

UV-Vis spectra of the DOM solutions were similar in all patches (data not shown). They showed the exponential decay with increasing wavelength commonly shown by soil organic matter (He et al. 2014). No significant differences in UV-Vis indices were found between patch and interpatch soils, except for SR, SUVA₂₈₀ and SUVA₂₅₄ (Table 2). According to Carder et al. (1989), the SR humification index can be employed to describe the relationship between humic and fulvic acids using a semi-quantitative approach. Thus, these results suggest that the content of humic acids may be higher in patch soils than in interpatch soils.

Table 2. UV-Vis indices for DOM in patch and interpatch soils. Different letters indicate significant differences between soils ($p < 0.001$)

	patch	interpatch
SR	0.9a	1.0b
SUVA ₂₈₀ (L mg ⁻¹ m ⁻¹)	2.5a	1.2b
SUVA ₂₅₄ (L mg ⁻¹ m ⁻¹)	4.0a	1.4b

Organic substances were small in size. He et al. (2014) found SR values of 0.7 in municipal solid waste, and this value decreased along the composting process, which indicates an increasing condensation of organic molecules. SUVA₂₅₄ and SUVA₂₈₀ were higher in patch soils respect to interpatch soils (Table 2). High SUVA₂₅₄ has been associated with high aromaticity (Peuravuori and Pihlaja 1997). Moreover, He et al. (2014), associated the absorption in the range 268-287 nm to lignin, and SUVA₂₈₀ to lignin degradation products, such as phenols. Sørensen (1962), found that abiotic decomposition of lignin resulted in brown, water soluble substances, especially when

the substrate was dry. Lignin is abundant in wood, and these molecules may be present in DOM. The smaller size of the molecules in interpatch soils is in agreement with the observed patterns of DOM in patch and interpatch soils, and the relationship between DOM and rainfall.

3.2.4. Organic acids

Retention times and UV-Vis spectra of the organic acids used as standards are shown in Fig. 5. Four different spectra were obtained for aliphatic acids without conjugated double bonds, aliphatic acids with conjugated double bonds, aromatic acids, and phenols (Fig. 4). In all cases, HPLC chromatograms of patch and interpatch soils, showed similar patterns (Fig. 5), with three main peaks that are not completely resolved. Peaks at 5.4 and 7.9 min, had an asymmetric shape, with long tails. The corresponding UV-Vis spectra (Fig. 6) were similar, as they showed a gentle slope towards long wavelengths and a clear shoulder in the region 270-280 nm. They may represent a mixture of various organic acids, or molecules with aromatic rings and aliphatic chains. The peak at 5.9 min was more defined, and was compatible with fumaric acid. Nambu et al. (2008) reported high concentrations of fumaric acid in soils derived from alkaline parent materials, as found in our study. Fumaric acid is an important plant metabolite and a sub-product of lignin degradation by fungi (Roy and Archibald, 1993). Total area, and the area of the three individual peaks were higher in patch soils than in interpatch soils. However, there was no significant relationship between these areas and TOC or DOC. A good relationship was observed between the total peak area less fumaric peak area (i.e. organic acids with aromatic structures), and $SUVA_{254}$ and $SUVA_{280}$ (Fig. 7).

3.3. Site effect

Campello soils contained less TOC and organic acids than soils from other sites (Table 3). DOC was lower in Aigües soils and higher in Tibi soils. Campello soils had the highest relative amount of DOC (DOC/TOC; Table 3).

Table 3. Amounts of DOC and TOC (C mg. soil g⁻¹) and DOC/TOC (mg.g⁻¹) ratio in patch and interpatch soils from four *Stipa tenacissima* steppes. Different letters indicate statistically significant differences between soil types (p<0.01).

	Aigües	Campello	Orihuela	Tibi
DOC				
patch	0.12b	0.20a	0.20a	0.22a
interpatch	0.07c	0.12b	0.12b	0.17a
TOC				
patch	80a	41c	55b	63b
interpatch	30a	16b	36a	40a
DOC/TOC				
patch	1.5b	5.7a	4.2a	4.0a
interpatch	2.9d	9.0a	3.5c	4.9b

Site also affected SOM properties measured by UV-Vis, and particularly those properties related to molecular size (Table 4). E2/E3 values were relatively high in all sites. High E2/E3 ratios indicate low molecular weights, and characterize organic molecules that can be easily transported (Guo and Chorover, 2003). All soils showed high values of E4/E6, which is characteristic of scarcely humified material which may contain proteins and carbohydrates. Likewise, E280/E472 indicates the relationship between low humified and partially humified organic molecules (Purmalis and Klavins,

2013). Aigües soils had the highest values of these indexes (Table 4). These results are consistent with the values of $\log\Delta k$ that, in all cases, corresponded to little humified substances (Cunha et al, 2009).

Table 4. UV-Vis indices for DOC in soils of the four *S. tenacissima* steppes studied.

Different letters indicate statistically significant differences between soil types ($p < 0.01$).

	Aigües	Campello	Orihuela	Tibi
<hr/>				
E2/E3				
patch	8.5a	4.6b	5.0b	5.0b
interpatch	11a	4.7b	4.72b	5.00b
<hr/>				
E4/E6				
patch	50a	30ab	14b	12b
interpatch	150a	34b	11c	12c
<hr/>				
SR				
patch	0.92a	0.74b	0.90a	0.92a
interpatch	1.05a	0.87b	1.09a	0.91ab
<hr/>				
E280/E472				
patch	22a	19a	19a	18a
interpatch	22a	22a	16b	18ab
<hr/>				
E270/E400				
patch	8.2a	7b	7.4b	7.6b
interpatch	9.3ab	11a	7.1b	8.0b
<hr/>				
$\log\Delta k$				
patch	1.38a	1.29a	1.19b	1.11b
interpatch	1.49a	1.25a	1.08b	1.10b
<hr/>				

Variability in SOM properties was related to climatic variables such as average temperature and average rainfall (Table 5). Correlation coefficients and stepwise regression showed similar results (data non shown). Thus, humification decreased as rainfall increased. This relationship was observed along the year, except in September when high temperatures and rain had the opposite effect. Similarly, low molecular weight molecules increased as average temperature increased. E280/E472, E280/E664, E474/E664 represent molecular relationships between low (E280); medium (E472) and high (E664) degree of humification (Purmalis and Klavins, 2013).

Table 5. Correlation coefficients between soil organic matter properties and climatic conditions. Only significant coefficients are shown ($p < 0.05$).

	<i>E2/E3</i>	<i>E4/E6</i>	<i>SR</i>	<i>E280/E472</i>	<i>E280/E664</i>	<i>E472/E664</i>	<i>E270/E400</i>	<i>Δlogk</i>	<i>DOC/TOC</i>
Altitude (m)				-0.435			-0.504		-0.597
Precipitation (mean)	0.852	0.505		0.472	0.586	0.593	0.323	0.658	
Precipitation (max)	0.565	0.344		0.476	0.374	0.379	0.355	0.467	
T (°C)	-0.864	-0.401			-0.589	-0.585	-	-0.541	0.375
T (°C) min									0.353
T(°C)max	-0.434								
Utmx	0.525	0.384		0.570	0.352	0.362	0.481	0.505	0.302
Utmy	0.562	0.356		0.502	0.374	0.380	0.385	0.480	
Rock	-0.533				-0.353	-0.343			0.407
Precipitation jan	0.548				0.359	0.356		0.351	
Precipitation feb	0.556	0.314		0.422	0.366	0.368		0.433	
Precipitation sep	-0.815	-0.391	-0.320		-0.567	-0.566		-0.509	0.437
Precipitation oct	0.565	0.344		0.476	0.374	0.379	0.355	0.467	
Precipitation nov	0.595	0.333		0.425	0.394	0.396		0.457	
Precipitation dec	0.700	0.378		0.419	0.468	0.470		0.515	
T°C (dif max-min annual)	-0.389	-0.350		-0.577	-0.263	-0.277	-0.531	-0.450	

Concerning organic acids, a peak with 10-minutes retention time appeared in soils from Tibi and Orihuela. Its UV-Vis spectra is characteristic of carboxylic with aromatic rings/phenol acids (Fig 5C, D; Fig. 8). In Orihuela, the aromatic peak appeared in patches of *Q. coccifera* and *R. lycioides*. In contrast, in Tibi the peak was also found under *P. lentiscus* and *J. oxycedrus*, and even in some interpatch soils.

3.4. Effect of dominant species on SOM properties

SOM underneath patches dominated by different species was similar, which is in agreement with other studies (Aranda and Comino, 2014). Yet, *E. fragilis* differed from other species in terms of TOC content UV-Vis properties (Table 6). Higher values of E2/E3, SUVA₂₅₄ and SUVA₂₈₀ in this species compared to other patch-forming species suggest greater incorporation of lignin. These increases may reflect changes in TOC, but also changes in DOM composition, as their magnitude was larger. Yet, these results must be taken with care because only four patches were dominated by *E. fragilis*, and they were all located in a single site (Aigües). Yet, they suggest a particular role of this species that may be worth exploring. In contrast, only *R. lycioides* showed differences in fumaric acid concentration (Fig. 9). This may result from higher production of fumaric acid by this species, or higher lignin degradation in the soil underneath patches dominated by *R. lycioides*.

Table 7: Mean values for soil organic matter and UV-Vis indices of DOC, related to the dominant species in woody patches of *Stipa tenacissima* steppes. Different letters indicate statistically significant differences between soil types ($p < 0.01$).

	<i>Ephedra</i>	<i>Juniperus</i>	<i>Pistacia</i>	<i>Quercus</i>	<i>Rhamnus</i>
	<i>fragilis</i>	<i>oxycedrus</i>	<i>lentiscus</i>	<i>coccifera</i>	<i>lycioides</i>

TOC (C mg. soil g ⁻¹)	95a	60b	46b	52b	52b
DOC (C mg. soil g ⁻¹)	0.21a	0.14a	0.18a	0.16a	0.15a
DOC/TOC (mg. g ⁻¹)	3.9a	3.0a	4.9a	3.9a	3.7a
E2/E3	18.8a	5.2b	5.5b	5.6b	5.7b
SUVA ₂₅₄ (L mg ⁻¹ m ⁻¹)	13.1a	3.3b	2.5b	3.2b	2.9b
SUVA ₂₈₀ (L mg ⁻¹ m ⁻¹)	3.8a	2.4b	2.2b	2.6b	2.5b

We found substantial differences in the fumaric acid content between patches dominated by *R. lycioides* and those dominated by other shrub species (Fig. 9). This may result from higher production of fumaric acid by the former species, or may be a consequence of higher levels of lignin degradation.

4. Conclusions

Woody patches greatly increased soil organic matter content, particularly in soils poor in organic C. The highest differences between patch and interpatch soils were related to the amount of organic matter. Although not all polar molecules were separated and identified by HPLC, the use of a UV-Vis detector allowed us to characterize groups of molecules with similar chemical characteristics. DOC represented a small fraction of these organic molecules and its content decreased with rainfall. Although the DOC may be leached from these soils, most TOC will remain. These soils act as carbon sinks and especially promising is the case of *Ephedra fragilis*. Despite substantial differences in TOC content, soils underneath woody patches showed scarce differences in DOC

quality, compared to interpatch soils. According to UV-Vis spectra, patches increased the degree of humification and OM aromaticity, and decreased molecular size. Likewise, species effect on TOC and DOC quality was weak, with the exception of *E. fragilis*. Further studies are needed to explore the impact of this species on SOM dynamics and storage.

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Figures

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Fig. 9

Figure captions

Figure 1. Principal components analysis describing organic matter properties in patch and interpatch soils in four *Stipa tenacissima* steppes (Aigües, Campello, Orihuela and Tibi). Site names are followed by “p” (for patch soils) or “i” (for interpatch soils). See text for description of SOM indices.

Figure 2. Relationship between TOC and DOC content in patch and interpatch soils

Figure 3. Changes in soil DOC content with annual rainfall in patch and interpatch soils in semi-arid steppes. Linear regression models are shown for both types of soil.

Figure 4. UV-Vis spectra models for several organic acids usually present in soils (standard solutions).

Figure 5. HPLC chromatogram for patch and interpatch soils in a *Stipa tenacissima* steppe.

Figure 6. UV-Vis spectra for the main peaks in Figure 5.

Figure 7. Relationship between the quantity of organic acids with aromatic rings (measured as peak area in 42 chromatograms) and SUVA₂₅₄ and SUVA₂₈₀ indices.

Figure 8. Characteristic UV-Vis spectra of the HPLC peak at 10 min retention time, of Tibi and Orihuela soils.

Figure 9. Fumaric acid concentration in patches with different dominant species and other patch characteristics. Different letters indicate statistically significant differences between both soils ($p < 0.05$)