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Humus quality in Terrra rossa soils under olive groves with different soil management types

Kvaliteta humusa u crvenicama pod maslinicima s različitim načinima gospodarenja tlom

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ABSTRACT

The paper aimed to determine humus quality in Terra rossa soils under olive groves with different soil management types. A total of 10 top-soil samples (0-20 cm) were collected in olive groves of Middle Dalmatia, Croatia, out of which 5 were from the traditional low-input olive groves (TOG) and 5 in the intensive olive groves (IOG). The soil samples were analyzed for basic soil properties, soil organic carbon (SOC), and fractional composition of humic substances. Spectroscopic characterization of humic substances was carried out by measuring absorbance in the VIS spectral range (400-700 nm). Optical index E_4/E_6 (ratio of optical absorbance at 465 to 665 nm for humic substances in solution) was calculated. The mean value of SOC in soils under TOG (3.06%) was lower than in soils under IOG (3.88%). Higher variations of carbon in humic (C_{HA}) and fulvic acid (C_{FA}) were observed in the soils under IOG. The mean C_{HA}/C_{FA} ratio in soils under TOG was higher than in soils of IOG (1.78 and 1.26, respectively). The soils under TOG had fulvic-humic to humic types, while the soils of IOG had humic-fulvic to humic types of humus. A lower mean E_4/E_6 index of soils under TOG than IOG (3.78 and 4.36, respectively) confirmed the results of the classical analytical method. Our findings reveal higher variation and lower humus quality in soils under intensive olive cultivation.

Keywords: Dalmatia, E_4/E_6 index, fulvic acid, humic acid, VIS spectroscopy

SAŽETAK

Cilj rada bio je utvrditi kvalitetu humusa u crvenicama pod maslinicima s različitim načinom gospodarenja tlom. Ukupno je uzeto 10 površinskih uzoraka tla (0-20 cm) iz maslinika Srednje Dalmacije, Hrvatska, od čega 5 iz tradicionalnih maslinika (TM) i 5 iz intenzivnih maslinika (IM). Uzorci tla su analizirani na osnovna svojstva tla, organski ugljik (OC) i frakcijski sastav humusnih supstanci. Spektroskopska karakterizacija humusnih supstanci provedena je mjerenjem absorbance u VIS spektralnom rasponu (400-700 nm). Izračunat je optički indeks E_4/E_6 (odnos optičke apsorbance kod 465 prema 665 nm za humusne supstance u otopini tla). Srednja vrijednost OC u tlima pod TM (3,06%) bila je niža nego u tlima pod IM (3,88%). Veća variranja ugljika u huminskim (C_{HA}) i fulvo (C_{FA}) kiselinama uočena su u tlima pod IM. Prosječni C_{HA}/C_{FA} odnos u tlima pod TM (1,78) bio je viši nego u tlima pod IM (1,26). Tla pod TM imala su fulvično-huminski do huminski tip, dok su tla pod IM imala huminsko-fulvični do huminski tip humusa. Niža srednja vrijednost E_4/E_6 indeksa u tlima pod TM (3,78) u odnosu na IM (4,36) potvrdila je rezultate klasične analitičke metode. Naši rezultati otkrivaju veće variranje i nižu kvalitetu humusa u tlima pod intenzivnom maslinarskom proizvodnjom.

Ključne riječi: Dalmacija, E_4/E_6 indeks, fulvo kiseline, huminske kiseline, VIS spektroskopija

INTRODUCTION

The content and quality of soil organic matter are the most important factors of soil quality and fertility (Stevenson, 1994) that has long been recognized by agronomist and policymakers. Organic matter is essential in the stabilization of soil aggregates, water infiltration, and erosion control (Aranda and Comino, 2014), retains nutrients as well as pollutants in the soil that improves plant growth and protects water quality (Lal et al., 2004), enhances biological diversity (Biswas and Kole, 2017) and helps mitigate the greenhouse effect of environment (Jarecki and Lal, 2005). Therefore, the study of the soil organic matter is vitally important for the sustainable management of agricultural land.

Soil organic matter is a heterogeneous mixture of organic compounds that differ in stability, decomposition degree, and turnover rate (Huang et al., 2008). Humic substances are generally classified into humic acid, fulvic acid, and humin based on their solubility in water as a function of pH (MacCharthy et al., 1990). The humic acid fraction consists of hydroxyphenol, hydroxybenzene acids, and other aromatic structures linked by peptides, amino compounds, and fatty acids. Fulvic acids are usually composed of various phenolic and benzene carboxylic acids linked by hydrogen bonds. Humin is considered an insoluble fraction (Schnitzer and Khan, 1975). Domination of more "mature" and stable humic acids in relation to fulvic acids indicates higher humus quality.

Numerous methods have been developed for the isolation, purification, and quantification of humic substances (van Zomeren and Comans, 2007). The classical methods of fractionation of humic substances (HS) are based on their differences in solubility in aqueous solutions (Kononova, 1966). The method of Schnitzer (1982) with a mixture of 0.1 M NaOH and 0.1 M $Na_4P_2O_7$ as the extractant is successful in maximizing extraction of humic compounds and minimizing their degradation. Also, it is suitable for all soil types regardless of carbonate content.

To determine molecular structure and chemical properties of humic substances non-destructive spectroscopic methods are commonly used (Chen et al., 2002; Pospišilova et al., 2008). The spectrometry approach includes a wide variety of techniques such as UV-VIS (Fuentes et al., 2006; Szajdak et al. 2007), SFS (Fasurova and Pospišilova, 2011), FTIR (Shirsova et al., 2006; Seddaiu et al., 2013) and ^{13}C-NMR (Abakumov et al., 2015). Humic substances generally show strong absorbance in the UV–VIS range (from 190 to 700 nm), due to the presence of aromatic chromophores and other organic compounds (Schnitzer and Khan, 1975). Absorption of humic substances on the wavelength of 465 nm is equal to the absorption of components linked to initial phases of humification (fulvic acid) and absorption of light on 665 nm refers to well-humified components (humic acid), Stevenson (1982). The optical index E_4/E_6 calculated as a ratio of the absorbances at 465 and 665 nm has been widely used to study humic substances (Szajdak et al., 2007; Aranda et al., 2011; Bensa et al., 2016; Vicente-Vicente et al., 2015; Mbarek et al., 2020). It is inversely related to the degree of condensation and aromaticity of the HS and their degree of humification (Stevenson, 1994). In general, lower molecular weight and lower degree of condensation of aromatic structures in humic substances show higher values of E_4/E_6, Orlov (1985).

Soils of the Mediterranean region are particularly susceptible to the loss of organic matter due to high temperatures and low rainfall, exposure to erosion, torrential storms, and wildfires that frequently occur in this region (Certini et al., 2011). In addition, these soils are vulnerable to management (Eswaran et al., 1999), including tillage and the use of mineral fertilization and pesticides. The depletion of soil organic matter content in Mediterranean soils under conventional farming systems in comparison to organic farming has been proven in many studies (Aranda et al., 2011; Parras Alcantara et al., 2013; Parras Alcantara and Lozano Garcia, 2014; Milgroom et al., 2007). Furthermore, some studies have addressed the impact of individual agricultural practices on soil organic matter composition of soils in the Mediterranean region, e.g. tillage (Laudicina et al., 2014; Alvaro–Fuentes et al., 2008; Blanco Moure et al., 2016), application of organic amendments (Brunetti et al., 2005; Perez Lomas et al., 2010; Aranda et al., 2015; Mbarek et al., 2020), crop rotation (Angeletti et al., 2021) and cover crops (Peregrina et al., 2012; Vicente-Vicente et al., 2015). However, restricted information is available on the effect of soil management on humus composition in a typical Mediterranean soil - Terra rossa (Bensa et al., 2016).

This study has been performed in one of the largest olive-growing areas of Croatia. It is located in Middle Dalmatia, a typical Mediterranean karst region characterized by a high density of specific dry-wall enclosures comprising mainly Terra rossa soil. This region is known for its long-lasting and diverse anthropogenic impact on landscapes and soils through olive growing and viticulture. Different types of olive growing involve different intensities of anthropogenic impact on soil properties.

We hypothesized that Terra rossa soils differ in humus quality depending on the soil management in olive groves. The goals of this study were to (i) determine humic substance (HS) composition, (ii) spectroscopically characterize HS and (iii) evaluate humus quality in Terra rossa soils under traditional low-input and intensive olive groves.

MATERIAL AND METHODS

Study area

The study was conducted in the olive growing area Marina in the Middle Dalmatia, Croatia. The region is characterized by a Mediterranean climate (Csa) with dry and hot summers and mild rainy winters (Filipčić, 1998), also known as the "olive climate". The study area is built of Cretaceous thin and thickly layered limestones and dolomites (Marinčić et al., 1971) characterized by typical karst geomorphology. The high fragmentation of land, numerous terraces, and dry-wall enclosures are distinctive features of this area. Rectangular dry stone enclosures known as "vlačice" are adapted to the terrain and stone configuration (Andlar et al., 2018). According to the Basic Soil Map of Croatia at a scale of 1: 50 000, section Šibenik 3 (Čolak and Martinović, 1974a) and Šibenik 4 (Čolak and Martinović, 1974b) dominant soil type is Terra rossa. The correlation analogue of mentioned soil type according to the IUSS Working group of WRB (2015) is Leptic Chromic Cambisols (Clayic). It is reddish, clayey, or silty clayey soil found covering limestones and dolomites in the karst Mediterranean region as a discontinuous layer with variable depth. The olive growing in the study area characterizes dry farming, including traditional low-input and intensified growing systems. Broadly spaced (scattered) old trees designated in the low-input growing system are rarely fertilized or treated with pesticides, as well as infrequently cultivated. The intensified olive growing system includes more intense fertilization (mineral and organic), plant protection and regular soil tillage.

Soil sampling and laboratory analysis

A total of 10 top-soil samples (0-20) cm were collected as composite samples in currently productive private farms in the study area during July 2022. The composite soil samples were taken by a pedological probe with a diameter of 80 mm. They consisted of 5 sub-samples taken at a distance of 1 m in a cross arrangement. Five soil samples were taken in olive groves under the traditional low-input system, and the other five from olive groves with the more intensive growing system.

The traditional low-input growing system characterizes density plantation of 70-200 trees/ha. These olive groves are located on hard–to–access rocky terrain where it is difficult or impossible to use agricultural machinery. The shallow soil tillage (up to 10 cm) is done manually and infrequently (mostly in autumn). Herbicides are not used. Fertilization is also rare and carried out with low doses of mineral fertilizers, and sometimes it is omitted. In a more intensive olive growing system, the density plantation is 250-500 trees/ha. Soil tillage is carried out mechanized and several times a year (deep in autumn, shallow in spring and very shallow in summer). The inter-row space is treated with herbicides. Fertilization is most often carried out by complex fertilizers (e.g. NPK 7-20-30 or 6-18-36) in autumn and with nitrogen (e.g. UREA) in spring. Usual annual fertilization amounts 150 kg N/ha, 60 kg P_2O_5/

ha, and 100 kg K_2O/ha. Furthermore, different organic amendments are also applied in various doses.

The disturbed soil samples were air-dried, grinded, and sieved using a sieve with a 2 mm mesh size (ISO 11464:2006). Soil particle size distribution was analysed by pipette-method (wet sieving and sedimentation) using sodium-pyrophosphate ($Na_4P_2O_7$, c=0.1 M) as a dispersant (ISO 11277:2009). Soil pH was measured using a combined glass electrode in a 1:5 (*v/v*) suspension of soil in water, and soil in KCl solution (c=1 M) according to ISO 10390:2005. Carbonate content was determined by a modified volumetric method (ISO 10693:1995). Physiologically available phosphorus and potassium were determined according to Egner-Riehm-Domingo lactate-acetate as the extraction solution (Egner et al. 1960). The humus content was analysed by acid potassium-dichromate ($K_2Cr_2O_7$, c=0.4 N) digestion, following the method of Tjurin (JDPZ, 1966). The humus content was then divided with the Van Bemmelen factor (1.724) to calculate soil organic carbon (SOC).

The isolation of soil humic substances (HS) was made by the Schnitzer method (1982). 5 g of air-dried soil sample was sieved at the mesh size of 1 mm and extracted with a solution of 0.1 M NaOH + 0.1 M $Na_4P_2O_7$. The mixture was shaken mechanically for 24 hours at room temperature. The supernatant solution was then separated from the residue by centrifugation at 4000 rpm for 20 min. The alkaline extract was acidified with concentrated H_2SO_4 to pH~1 and allowed to stand for 24 h at room temperature to obtain the complete precipitation of humic acid (HA). The precipitated HA was separated from fulvic acid (FA) by repeating three times the following: centrifugation at 4000 rpm for 20 min, removal of the residue, and washing the HA with 0.05 M H_2SO_4 solution. Finally, the centrifuged HAs were dissolved in a minimal volume of 0.1 M NaOH and brought to dryness in a drying oven at 60 °C. The sums of HS, HA, and FA were determined by the titrimetric method in aliquot volumes. The VIS spectra were measured by Shimadzu UV 1700 spectrometer in the range of 400-700 nm. The extracts were prepared as a mixture of 0.1 M NaOH and 0.1 M $Na_4P_2O_7$.

Statistical analysis

Descriptive statistics of basic soil properties (soil fractions content, pH, $CaCO_3$, P_2O_5, K_2O, SOC) and humic substances (humic and fulvic acid) included mean, median, standard deviation (SD), coefficient of variation (CV) and skewness.

RESULTS AND DISCUSSION

Basic soil properties

Particle size distribution analysis of the soil samples from traditional olive groves (TOG) revealed domination of fine silt particles followed by clay particles (mean values 45.1 and 32.0%, respectively) (Table 1). The soils under intensive olive groves (IOG) are also fine-textured but contain fewer fine silt particles and more clay (mean value 37.4 and 37.9%, respectively) (Table 1). The total content of sand (coarse and fine) is negligible in both groups of samples.

The studied soils are silty clay loams, except for one sample of soil under IOG that belongs to the silty clay texture class. Obtained results are typical for Terra rossa soils proven in many studies (Bellanca et al., 1996; Vingiani et al. 2018; Miloš and Bensa, 2020; Miloš and Bensa, 2021).

Studied soils under both management types of olive production have slightly acidic to neutral reactions, which is in accordance with previous studies of Terra rossa soils (Peh et al., 2003; Conde et al., 2007; Vingiani et al., 2018). Still, pH_{KCl} in soils of TOG varies in a narrower range (6.48 to 6.90) compared to the range in soils under IOG (5.62-7.19) (Table 2). The distribution of pH_{KCl} in soils under TOG is approximately symmetric (skew -0.02), while in soils under IOG, it is highly negatively skewed (skew -1.64). Higher variability of pH in soils under IOG can be attributed to the impact of different mineral fertilizations in olive groves with an intensive growing system. Furthermore, soils under TOG are slightly calcareous with $CaCO_3$ content in the range of 1.5-3.0%, while soils under IOG are slight to moderately calcareous having a wider range of $CaCO_3$ content (2.2 to 19.3%; Table 2).

Although typical Terra rossa soils formed "in situ" are non-calcareous (Škorić et al., 1985; Peh et al., 2003), a colluvial subtype of Terra rossa soil contains carbonates due to the presence of carbonate skelet (Bogunović et al., 2009). Some studies of Terra rossa of Middle Dalmatia (Miloš and Maleš, 1998; Miloš and Bensa, 2020) also reported a similar range of $CaCO_3$ content and attributed it to the process of colluviation.

Table 1. Descriptive statistics for particle size distribution of the studied soil samples

Management type	Soil fraction (%)*	Min	Max	Mean	Med	SD	CV	Skew
Traditional (TOG)	Coarse sand	0.1	0.7	0.3	0.2	0.22	66.0	0.83
	Fine sand	1.9	3.1	2.5	2.6	0.40	16.0	-0.22
	Coarse silt	17.8	22.3	20.0	19.7	1.55	7.8	0.20
	Fine silt	42.8	50.2	45.1	44.3	2.63	5.8	1.86
	Clay	27.6	35.0	32.0	32.3	2.48	7.7	-1.14
Intensive (IOG)	Coarse sand	0.2	10.6	2.7	0.7	3.98	14.83	2.19
	Fine sand	1.7	4.0	2.8	2.7	0.82	29.8	0.31
	Coarse silt	18.0	21.2	19.3	18.4	1.44	7.5	0.58
	Fine silt	35.7	42.3	37.4	36.4	2.50	6.7	2.10
	Clay	31.6	44.0	37.9	37.7	4.07	10.7	-0,05

*Coarse sand (2.0-0.2 mm); Fine sand (0.2-0.063 mm); Coarse silt (0.063-0.02 mm); Fine silt (0.02-0.002 mm); Clay (< 0.002 mm).
Min – minimum; Max – maximum; Med- median; SD – standard deviation; CV – coefficient of variation; Skew- skewness.

Table 2. Descriptive statistics for basic chemical properties of the soil samples

Management type	Soil property	Min	Max	Mean	Med	SD	CV	Skew
Traditional (TOG)	pH_{H_2O}	7.21	7.75	7.47	7.48	0.21	2.7	0.04
	pH_{KCl}	6.48	6.90	6.70	6.67	0.15	2.3	-0.02
	$CaCO_3$ (%)	1.5	3.0	2.0	1.7	0.58	29.0	1.15
	P_2O_5 (mg/kg)	9.0	14.6	12.9	13.7	2.1	0.16	-1.30
	K_2O (mg/kg)	255	490	331	265	92.6	0.28	1.20
Intensive (IOG)	pH_{H_2O}	6.51	7.92	7.34	7.41	0.47	6.5	-0.98
	pH_{KCl}	5.62	7.19	6.65	6.91	0.55	8.2	-1.64
	$CaCO_3$ (%)	2.2	19.3	5.74	2.3	6.78	11.8	2.23
	P_2O_5 (mg/kg)	255	494	340	270	97.3	0.29	0.89
	K_2O (mg/kg)	591	2520	1155	869	693.9	0.60	2.05

Min – minimum; Max – maximum; Med- median; SD – standard deviation; CV – coefficient of variation; Skew- skewness

The differences in the content of plant available nutrients between studied soils were even more pronounced. The soils under TOG are poorly supplied with physiologically available phosphorus (9.0-14.6 mg/kg), while soils of IOG contain much higher concentrations of P_2O_5 (255-494 mg/kg) (Table 2). Likewise, the concentrations of physiologically active potassium in soils under TOG are significantly lower than in soils under IOG (mean values 331 and 1155 mg/, respectively). Obtained results are in line with the study of Miloš and Bensa (2020) that reported low content of P_2O_5 and medium of K_2O in Terra rossa of Dalmatia under natural vegetation. The authors pointed out that it is typical for all soil derived from limestones and dolomites.

SOC and humic substances composition

SOC content and fractional compositions of humic substances for each sample of studied soils are presented in Table 3 and descriptive statistics for analyzed properties are in Table 4. The SOC content in soils under TOG varied from 2.52 to 3.54%, while in soils under IOG was in the range of 3.39-4.08% (Table 3). The mean value of SOC content in soils under TOG (3.06%) is lower than in the soils under IOG (3.88%) (Table 4). The soils under TOG have an approximately symmetric distribution of SOC (skew -0.41), while in soils under IOG, it is highly negatively skewed (skew -1.98).

The established SOC values in soils under TOG correspond to previous findings for natural Terra rossa soils of Middle Dalmatia (Miloš and Bensa, 2017; Miloš and Bensa, 2021). It implies minimal anthropogenic impact in studied soils under TOG. Higher mean value and asymmetric distribution of SOC values in the soils under IOG can be attributed to different organic fertilizations applied in olive groves with more intensive growing systems. Numerous authors (Brunetti et al., 2005; Perez Lomas et al., 2010; Aranda et al., 2015; Mekki et al., 2017; Mbarek et al., 2020) have reported an increase in SOC content after the application of organic amendments. On the other hand, soil tillage stimulates mineralization and reduces SOC content (Haddaway et al., 2017; Hashimi et al., 2022).

Hence, some studies (Hernandez et al., 2005; Aguilera–Huertas et al., 2022) reported SOC loss in soils of a Mediterranean region under olive groves with conventional tillage. Mbarek et al. (2020) stressed that exposure of organic matter to oxidation by tillage combined with the aridity of the Mediterranean climate accelerates the mineralization of organic matter. Busari et

Table 3. SOC and fractional composition of humic substances of studied soils

Management type	Sample	SOC (%)	C_{HA} (%)	C_{FA} (%)	C_{HA}/C_{FA}
Traditional (TOG)	T1	3.45	1.15	0.42	2.74
	T2	3.24	0.84	0.50	1.68
	T3	2.52	0.62	0.49	1.27
	T4	2.56	0.72	0.37	1.95
	T5	3.54	0.74	0.59	1.25
Intensive (IOG)	I1	3.92	0.65	0.86	0.76
	I2	4.05	1.30	0.61	2.13
	I3	3.99	1.01	1.04	0.97
	I4	3.39	0.58	0.67	0.87
	I5	4.08	1.06	0.67	1.58

C_{HA} – carbon in humic acid; C_{FA}- carbon in fulvic acid al. (2015) attributed the loss of SOC in tilled soils to soil structure deterioration and boosted erosion. However, organic fertilization can compensate for this loss and increase SOC content (Mbarek et al., 2020). Perez Lomas et al. (2010) pointed out that the lower the SOC content in the original soil, the higher the increase after compost application.

The carbon in humic substances (C_{HA}) of soils under TOG varied between 0.62 and 1.15%, while carbon in fulvic acids (C_{FA}) was in the narrower range of 0.37-0.59% (Table 3). The C_{HA}/C_{FA} ratio was in the range of 1.25 – 2.74 which points to fulvic-humic (C_{HA}/C_{FA} 1.0-1.5) to humic type (C_{HA}/C_{FA} > 1.5) of humus, according to Hristov and Filcheva (2017). Soils under IOG had C_{HA} content in a similar range (0.58 -1.30%). However, the C_{FA} content had higher values and varied in a wider range (0.61 – 1.04%) than in soils under TOG. The C_{HA}/C_{FA} ratio in soils under IOG varied from 0.76 to 2.13 (Table 4). The prevalence of fulvic acids over humic acids in samples I1, I4, and I5 points to the humic-fulvic type of humus (C_{HA}/C_{FA} 0.5-1; Table 3), while samples I2 and I5 have a humic type of humus (Hristov and Filcheva, 2017).

The descriptive statistic for C_{HA} and C_{FA} content in studied soils reveals higher variation in soils under IOG (CV 29.2 and 20.7, respectively) compared to soils under TOG (CV 22.4 and 15.8, respectively) (Table 4). Distributions of C_{HA} and C_{FA} in soils under TOG are approximately symmetric (skewness 0.18 and 0.21, respectively). The distribution of C_{HA} in soils under IOG is also approximately symmetric (skewness -0.01), but C_{FA} content has a highly positively skewed distribution (Skew 1.06) (Table 4). These data point to more uniform humus quality in soils under TOG. The mean and median values of the C_{HA}/C_{FA} ratio in soils under TOG (1.78 and 1.68, respectively) are higher than in soils of IOG (1.26 and 0.97, respectively) (Table 4). The C_{HA}/C_{FA} ratio expresses the degree of evolution of the humification process and indicates humus quality. Therefore, these results indicate higher humus quality in soils under TOG than in IOG.

In general, the obtained results are in line with the study of Hristov and Filcheva (2017) that reported humic and fulvic-humic types of humus for Bulgarian Cambisols with a dry and warm climate. Lopez–Pineiro et al. (2007) also found C_{HA}/C_{FA} ratios > 1 in leptic Cambisols of Portugal. However, Fasurova and Pospišilova (2011) determined lower C_{HA}/C_{FA} ratios (0.54-0.60) for Cambisols in the Czech Republic.

Higher humus quality in soils under TOG than in IOG in the current study can be attributed to the absence of tillage. Tillage is unfavorable for the process of humification and the formation of more stable humic substances. Several studies (Murage and Voroney, 2008;

Table 4. Descriptive statistics of SOC and analysed properties of humic substances in studied soils

Management type	Soil property	Min	Max	Mean	Med	SD	CV	Skew
Traditional (TOG)	SOC (%)	2.52	3.54	3.06	3.24	0.44	14.3	-0.41
	C_{HA} (%)	0.62	1.15	0.81	0.74	0.18	22.4	0.18
	C_{FA} (%)	0.37	0.59	0.47	0.48	0.07	15.8	0.21
	C_{HA}/C_{FA}	1.25	2.74	1.78	1.68	0.55	30.8	1.12
Intensive (IOG)	SOC (%)	3.39	4.08	3.88	3.99	0.25	6.5	-1.98
	C_{HA} (%)	0.58	1.30	0.92	1.01	0.27	29.2	-0.01
	C_{FA} (%)	0.61	1.04	0.77	0.67	0.16	20.7	1.06
	C_{HA}/C_{FA}	0.76	2.13	1.26	0.97	0.52	41.3	1.01

Min – minimum; Max – maximum; Med- median; SD – standard deviation; CV – coefficient of variation; Skew- skewness

Slepetiene et al., 2010; Seddaiu et al., 2013) reported an increase of humic acids in less-tilled soils under different land use. On the contrary, Aranda et al. (2011) noted a slightly higher C_{HA}/C_{FA} ratio in soils on limestones that were conventionally tilled in comparison to organically managed olive groves (1.1 and 1.3, respectively).

Another crucial factor that affects humus quality is fertilization. Although data on applied doses and types of fertilization are not available in the current study, the wide range of plant-available nutrients (Table 2) indicates differences among studied soils regarding mineral fertilization. The assumed differences in organic fertilization are noticeable in variable SOC content and the relationships between humus content and quality. Senesi et al. (1999) emphasized that the properties of humic substances in organic amendments determine their short-term and long-term effects on humic substances in soil depending on the origin and nature of the amendments. The samples I2 and I5 in the current study have the highest SOC contents (4.05 and 4.08%, respectively) and the highest humus quality (C_{HA}/C_{FA} 2.13 and 1.58, respectively) (Table 3). It indicates the application of high-quality organic amendments. Several studies reported an increase of C_{HA}/C_{FA} ratio after application of crop waste and manure (Dorado et al., 2003) and compost derived from recycling by-products of olive production (Kavvadias et al., 2018).

However, sample I1 in the current study has high SOC content (3.92%) but low C_{HA}/C_{FA} below 1 (0.76; Table 3). Increased SOC content suggests the application of organic amendments, but the prevalence of fulvic acids pointed to low humification degree. It is in agreement with the study of Perez Lomas et al. (2010) who reported an increase of humic acid (1.9 times) and fulvic acid (3.3 times) after compost application in soils under olive groves. Even though both humic substances increased C_{HA}/C_{FA} ratio decreased due to the greater increase of fulvic than humic acids. Lopez–Pineiro et al. (2007) also found a decrease in humification degree in Luvisols and Cambisols under olive groves in Portugal after the application of olive pomace. Brunetti et al. (2005) reported the increase of aliphaticity, O content, and acidic functional groups, and the decrease of N and C content in soil organic matter after application of olive pomace in a Mediterranean environment. The study of Mbarek et al. (2020) compared natural and tilled soils under olive groves (with and without organic amendments) in Tunis. The authors found the highest C_{HA}/C_{FA} (2.16) in natural soil and the lowest in cultivated soil without amendments (0.36). Cultivated soil with the application of organic amendments had significantly higher C_{HA}/C_{FA} (1.62) in comparison to tilled soil without amendments, but a lower than natural soil. The results in current study confirm different anthropogenic influences (tillage, fertilization) on humus quality observed in previous studies.

VIS spectroscopic characterization of humic substances

Various techniques of spectral characterization of humic substances in soil, such as UV–VIS, DRIFT, SFS, FTIR, and ^{13}C-NMR, are commonly used methods for identifying humus quality (Chen et al., 2002). Since humic substances show strong absorption in the UV-VIS range (190-700 nm) due to the presence of aromatic chromophores (Schnitzer and Khan, 1975), UV-VIS spectroscopy is convenient for evaluating the humification degree of different organic alkaline extracts (Fuentes et al., 2006). Therefore, UV-VIS spectroscopy is one of the most frequently used methods for humic substance characterization (Shirshova et al., 2006).

The VIS absorption spectra of humic substances extracted from studied soils show a monotonous decrease of the absorption from 400 to 700 nm (Figure 1).

In general, a steeper decline in the curves was observed for soils under TOG compared to IOG. The steeper decline implies the domination of aromatic over aliphatic structures (Pospíšilová and Fasurová, 2009; Fasurová and Pospíšilová, 2011; Milori et al., 2002). It is in agreement with the results obtained by the classical analytical method (Tables 3 and 4).

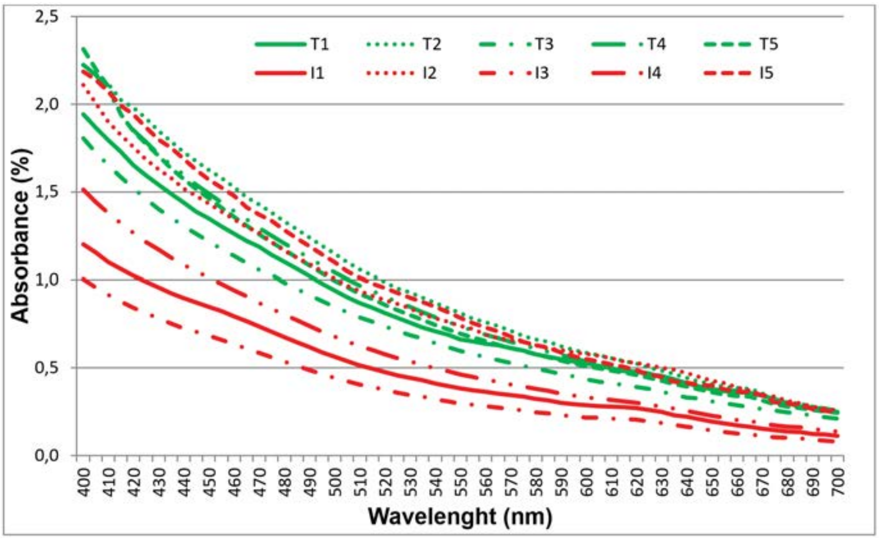

Figure 1. VIS spectra of extracted humic substances in studied soils

The absence of any well-defined UV-Vis minima and maxima feasibility results from the extended overlap of absorbances of a wide variety of chromophores affected by various substitutions (Stevenson, 1994; Senesi and Lofredo, 1999). Despite these limitations, the optical index E_4/E_6, calculated as the ratio of optical absorbance at 465 to 665 nm for humic substances in solution, has been widely used to assess soil organic matter quality (Szajdak et al., 2007; Aranda et al., 2011; Reddy et al., 2014; Bensa et al., 2016; Vicente-Vicente et al., 2015). The absorption of humic substances on a wavelength of 465 nm refers to fulvic acids and the absorption of light on a wavelength of 665 nm to humic acid, Stevenson (1982). Therefore, E_4/E_6 index is negatively correlated to the content of condensed aromatic structures (Stevenson, 1994; Fuentes et al., 2006) and decreases as condensation, aromaticity, and molecular weight of humic substances increases.

The mean values of the E_4/E_6 index of humic substances isolated from soils under TOG and IOG were 3.78 and 4.36, respectively) (Table 5).

The E_4/E_6 index < 4 indicates the presence of more aromatic and fewer aliphatic compounds, and high humus quality. Many authors (Senesi et al., 1999; Chen et al., 2002; Reddy et al., 2014) assert that the E_4/E_6 index decreases when the molecule weight or degree of condensation increases. The E_4/E_6 indices for all soil samples from TOG were below 4 (3.42-3.97), while the soils under IOG were in the range of 3.52-5.05 (Table 5). Furthermore, the variability of the E_4/E_6 index was more pronounced in soils under IOG compared to TOG (CV 12.9 and 5.1, respectively) (Table 5). These results point to a higher and more uniform humus quality in soils under TOG.

Obtained results are in line with the previous studies of humus quality in Terra rossa soils of Dalmatia (Čolak and Martinović, 1974b) and Italy (Senesi et al.,1999) that found similar ranges of E_4/E_6 indices (3.8-5.0 and 4.6-4.9, respectively). Brunetti et al. (2016) have found higher values of the E_4/E_6 indices in Terra rossa soil of Italy (6.57-7.02) indicating slightly poorer humus quality.

Table 5. Descriptive statistics for $E_{4/6}$ index of humic substances in soil solution

Management type	Min	Max	Mean	Med	SD	CV	Skew
Traditional (TOG)	3.42	3.97	3.78	3.86	0.19	5.1	-1.54
Intensive (IOG)	3.52	5.05	4.36	4.63	0.56	12.9	-0.52

Min – minimum; Max – maximum; Med- median; SD – standard deviation; CV – coefficient of variation; Skew- skewness

Lower E_4/E_6 indices found in the soils under TOG agree with the study of Bensa et al. (2016) that proved higher humus quality in Terra rossa soils under olive groves with lower SOC content. Vicente-Vicente et al. (2015) also reported a positive correlation between SOC content and $E_{4/6}$ index for soils of Andalusian olive groves. Authors reported a higher E_4/E_6 index in soils under natural plant cover than in conventionally tilled olive groves. The higher proportion of aliphatic compounds in soil organic matter of olive groves with plant cover authors attributed to the incorporation of fresh soil organic matter. Garcia–Gil et al. (2004) reported an increase in the E_4/E_6 index due to the application of compost and attributed it to the properties of organic amendment. Aranda et al. (2014) found very similar E_4/E_6 indices in different soils of Mediterranean natural ecosystems in Spain though their SOC content differed significantly.

CONCLUSION

Studied soils under olive groves with different soil management differed in SOC content, humic substance composition, and humus quality. Traditional low-input olive groves had lower SOC content than soils under a more intensive olive growing system. The C_{HA}/C_{FA} ratios in all soil samples from traditional olive groves reveal a prevalence of humic over fulvic acids. In intensive olive groves domination of fulvic acids was observed in part of the samples, while in the others humic acid dominated. On average, the C_{HA}/C_{FA} ratio was higher in soils under traditional olive groves indicating higher humus quality. The VIS spectroscopic characterization of humic substances isolated from studied soils and the E_4/E_6 index confirm more uniform and higher humus quality in soils under traditional olive groves. Various data on humus quality in intensive olive groves point to the need for further, more detailed studies to improve the sustainability of this growing system in a fragile Mediterranean environment.

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