https://doi.org/10.17221/165/2020-PSE

Soil phenolic compound variability in two Mediterranean olive groves

Eleonora Grilli[1], Esterina Di Resta[1], Monica Scognamiglio[1], Severina Pacifico[1], Antonio Fiorentino[1], Thiago Assis Rodrigues Nogueira[2], Renata Concetta Vigliotti[1], Antonio Ganga[3]*

[1]*Department of Environmental, Biological and Pharmaceutical Science and Technologies, University of Campania "Luigi Vanvitelli", Caserta, Italy*
[2]*Department of Plant Protection, Rural Engineering and Soils, School of Engineering, São Paulo State University, São Paulo, Brazil*
[3]*Department of Architecture, Design and Urban Planning, University of Sassari, Polo Bionaturalistico, Sassari, Italy*
**Corresponding author: eleonora.grilli@unicampania.it*

Citation: Grilli E., Di Resta E., Scognamiglio M., Pacifico S., Fiorentino A., Nogueira T.A.R., Vigliotti R.C., Ganga A. (2020): Soil phenolic compound variability in two Mediterranean olive groves. Plant Soil Environ., 66: 207–215.

Abstract: Phenolic compounds (PCs) – with special reference to secondary plant metabolites – were characterised in two Mediterranean olive groves (*Olea europaea* L.). Representative pedological profiles were dug to identify and characterise the pedotype. Qualitative and quantitative analyses were carried out on soil core samples gathered at fixed depths (0–20 cm and 20–40 cm) and olive leaf methanol extracts by high-performance liquid chromatography with ultraviolet detection. The total PCs content reflected the soil organic carbon distribution, especially carbon of humic and fulvic acids, corroborating their crucial role in humification pathways. Among the analysed plant secondary metabolites, luteolin-4'-*O*-glucoside and verbascoside were the most abundant in leaves and soils, respectively. Most of the easily hydrolysed/metabolised phenols were not found in soils. Rutin and verbascoside, despite containing glucose, strongly persisted in the soil environment, probably due to their allelopathic effect. Oleuropein was not found in soils because it is highly soluble and mobile in the soil environment. Furthermore, the presence of clay in soil seemed to determine the accumulation of specific PCs. Our data suggest that PCs persistence in soil seems to be mainly determined by a balance between physicochemical and biochemical instability and allelopathic stability rather than their abundance in the plant.

Keywords: total phenols; water-soluble phenols; soil-olive system; flavonoid; degradation

Along with humic substances, phenolic compounds (PCs) represent the largest constituents and the most active fractions of soil organic matter (Muscolo et al. 2013). PCs present in vegetation as secondary plant metabolites are the key source of phenolic matter in soil (Li et al. 2010). Monomeric (flavonoids and phenolic acids) and polymeric (lignins and tannins) phenols are mainly released into the soil through three main pathways, namely (*i*) leaching from aboveground plant material; (*ii*) exudation from plant roots, and (*iii*) litter decomposition (Hättenschwiler and Vitousek 2000). In addition, phenolic secondary metabolites can be directly introduced to soil by microorganisms (e.g., algae, fungi, lichens, and mosses) (De Carvalho et al. 2016).

In soil, three main phenolic forms occur, namely (*i*) free or dissolved, which move freely in the soil solution; (*ii*) sorbed or reversibly bound, sorbed by clay minerals, or forming chelate complexes with proteins and/or metals; and (*iii*) polymerised as humic substances (Min et al. 2015). The form, rather than the chemical structure (Schmidt et al. 2011), determines the degradation rate of phenols in soil. In particular, dissolved PCs are degraded in soil by external phenoloxidase or peroxidase enzymes (Sinsabaugh 2010) produced by microorganisms such

https://doi.org/10.17221/165/2020-PSE

as fungi (e.g., Basidiomycetes and Ascomycetes) and bacteria (e.g., *Pseudomonas*) (Sugiyama and Yazaki 2014), while physically and chemically protected PCs can persist longer than dissolved forms (Min et al. 2015). This suggests that PCs in the soil-plant continuum undergo a continuous cycle of synthesis, deposition, decomposition, leaching, and chemical immobilisation (Makoi and Ndakidemi 2007). Soil phenols are largely influenced by vegetation (Malá et al. 2013), but their concentration is not directly related to their content in trees and ground vegetation (Kanerva et al. 2008). The types and concentrations of soil phenols depend on the plant species and various biotic and abiotic factors, such as the cultivation system, biotic stresses, environmental conditions, nutrient availability, and plant development (Cesco et al. 2012). Studies on the dynamics of phenolic matter in pedo-agrosystems, with particular reference to secondary plant metabolites, are of a great interest for various scientific fields, such as chemistry, ecology, biology, and pedology; however, current studies are lacking and frequently have contradictory results and conclusions (Chomel et al. 2016). Furthermore, few works focusing on the distribution of phenols along profiles of well-characterised and classified soils are available in the literature (Gallet and Pellissier 1997, Northup et al. 1998, Rimmer and Abbott 2011, Kaiser et al. 2014, Massaccesi et al. 2018). Both soil heterogeneity and phenolic properties (i.e., form and reactivity) make the determination of the amount, degradation, and turnover of secondary plant metabolites in soils difficult (Cesco et al. 2012).

This study aims to (*i*) evaluate the contents of PCs in two olive grove soil systems by characterising them with particular reference to olive leaf secondary metabolites and (*ii*) to increase our knowledge on their concentrations as well as persistence, degradation/turnover, and behavior along the soil-olive system.

MATERIAL AND METHODS

Site location and agricultural aspects

The study was carried out during January 2012 in two olive groves of *Olea europaea* L., cv. Sessana located in Sessa Aurunca (Caserta province, Campania region, Italy), which is a historical production area of high-quality olive oil. The study area is characterised by a Mediterranean oceanic to suboceanic climate (Costantini et al. 2004). From 2004 to 2012, average annual air temperature and mean annual precipitation were 15.8 ± 0.1 °C and 1 009.5 ± 13.3 mm, respectively. November and December are characterised by the heaviest rainfall, while July and August are the driest months (Regional Agrometeorological Centre). The soil moisture and temperature regimes are xeric and thermic, respectively (Costantini et al. 2004).

The two olive groves have a flat morphology and are approximately 100 years old. The Aconursi (ACO) olive grove (41°17'01.97''N, 7°54'20.51''E; 190 m a.s.l.) is 0.33 ha with a distance between the plants of 6 × 7 m (242 plants/ha) and is managed with natural and permanent green cover (mowing is conducted twice each year and the mowed grasses are left in place). The Lauro (LAU) olive grove (41°15'55.65''N, 7°52'49.64''E; 60 m a.s.l.) is 0.38 ha with a distance between the plants of 7 × 8 m (160 plants/ha), herbaceous covering is nonexistent.

In both olive groves, no deep plowing and irrigation are conducted, trees are pruned every 3 years and the chipped prunings are removed.

Soil sampling

Soil profiles. One representative pedological profile was dug in the central part of each olive grove to identify the pedotype. They were described morphologically using standard soil survey methodology (Schoeneberger et al. 2012) and classified according to WRB (IUSS Working Group 2015). Selected morphological and physico-chemical features of both soils are reported in Table 1. Both soils developed from products of Roccamonfina volcanic activity (Geological Survey of Italy 1966) and were classified as Leptic Andosols (Loamic) and Leptic Regosols (Loamic) (IUSS Working Group WRB 2015), Aconursi and Lauro olive grove, respectively.

Soil core samples. Soil core samples were gathered before mowing at fixed depths of 0–20 cm as topsoil and 20–40 cm as subsoil to identify typical olive phenols and to understand their evolution and differentiation in soils. Four olive trees were selected in the central part of each olive grove, and four soil throughfall samples (four topsoil and four subsoil samples) were collected for each tree at 70 cm from the trunk. Thirty-two soil core samples were collected and analysed for each olive grove.

Soil analysis

Soil analysis was conducted according to Italian official procedures (MiPAF 2000) and interna-

https://doi.org/10.17221/165/2020-PSE

Table 1. Selected physical and chemical properties of the Aconursi and Lauro olive grove soils

Olive grove	Depth (cm)	Texture				BD	VG (%)	pH_{H_2O}	pH_{KCl}	EC	TOC	TN	WPCs	TPCs	CEC	$Al_o + ½Fe_o$	PR
		sand	silt	clay	class[a]	(g/cm^3)		1:2.5		(dS/m)	(g/kg)		(g VA/kg)		($cmol_+/kg$)	(%)	
Aconursi																	
Ap	0–20/ 25	60.6	23.2	7.0	sl	1.20	38	6.9	5.2	0.299	19.7	1.19	0.06	1.20	15.29	0.68	58
A*/R	20/25–40/45	54.5	35.7	9.8	sl	nd	30	6.9	5.0	0.212	6.9	0.88	0.04	0.38	16.88	0.45	40
Lauro																	
Ap	0–27	48.8	38.4	12.8	l	1.12	12	6.5	4.9	0.213	7.9	0.89	0.04	0.56	14.90	0.20	16
B_w**/R	27–45	36.5	40.4	23.1	l	nd	9	6.5	4.7	0.248	6.5	0.81	0.03	0.39	14.49	0.17	12

[a]textural class: sl – sandy loam, l – loam; BD – bulk density; VG – volcanic glass; EC – electrical conductivity; TOC – total organic carbon; TN – total nitrogen; WPCs and TPCs – water-soluble and total phenolic compounds; CEC – cation exchange capacity; Al_o, Fe_o – Al and Fe extracted by ammonium oxalate; PR – phosphate retention; nd – not detectable; *values refer to A horizon; **values refer to B_w horizon

tional standards (Soil Survey Staff 2014). All the determinations were conducted in triplicate. Soil samples were air-dried and sieved with a 2 mm sieve. Sand, silt, and clay were separated by wet sieving and pipetting. Soil pH was measured potentiometrically in a soil/solution mixture of 1:2.5 H_2O or 1 mol/L KCl. Electrical conductivity was detected in a 1:5 soil:water extract. The total organic carbon (TOC) content was estimated by the Spring-Klee method. The wet chemical procedure proposed by Dell'Abate et al. (2002) and modified by Rubino et al. (2008) was used for the extraction and fractionation of different organic carbon pools, namely the carbon content in humic and fulvic acids (HA+FA-C) and in humin (HUM-C), the sum of which represents the total humic carbon (THC). Non humic carbon (NHC) was measured, too. Selective dissolution was performed in ammonium oxalate (Al_o and Fe_o). Phosphate retention and volcanic glass were measured according to the procedures described by Blakemore et al. (1987) and Soil Survey Laboratory Staff (1996), respectively.

Determination of total and water-soluble phenolic compounds. Soil PCs were extracted as total PCs (TPCs) and water-soluble PCs (WPCs) using a 0.1 mol/L NaOH solution and distilled water, respectively, as reported by Buondonno et al. (2014). They were quantified by the Folin-Ciocalteu colorimetric procedure using vanillic acid as a standard (Box 1983, Lowe 1993). The difference between TPCs and WPCs corresponds to PCs with a high affinity (HPCs) for the soil body in a physicochemical sense, which are often linked to clay particles and humic substances (Buondonno et al. 2014).

Determination and quantification of phenolic compounds

To better understand the soil phenolic dynamics, qualitative and quantitative analyses were conducted on soil core samples and olive leaf methanol extracts by high-performance liquid chromatography equipped with an ultraviolet detector (HPLC-UV). Olive leaves were ground in liquid nitrogen, freeze-dried and stored at –20 °C, while topsoil and subsoil samples were sieved with a 0.5 mm sieve. Soxhlet extraction was selected as the extraction method (Mahugo Santana et al. 2009). The Soxhlet extractions lasted 24 h. The resulting extracts were dried under a vacuum by a rotary evaporator (Heidolph Hei-VAP Advantage, Schwabach, Germany).

https://doi.org/10.17221/165/2020-PSE

Table 2. Chemical properties, soil organic carbon fractions and soil phenol compounds in Aconursi (ACO) and Lauro (LAU) soil core samples (TOP = 0–20 cm; SUB = 20–40 cm) (mean value ± standard error)

	pH_{H_2O} (1:2.5)	pH_{KCl} (1:2.5)	EC (dS/m)	Clay (%)	TN (g/kg)	TOC (g/kg)	HA+FA-C (g/kg)	HUM-C (g/kg)	THC (g/kg)	NHC (g/kg)	C:N	WPCs (g VA/kg)	TPCs (g VA/kg)	HPCs (g VA/kg)	THU (%)
ACO TOP (n = 16)	6.9 ± 0.04[a]	5.2 ± 0.04	0.27 ± 0.01[a]	8.1 ± 0.8	1.52 ± 0.35[a]	15.84 ± 0.23[a]	4.77 ± 0.17[a]	8.81 ± 0.33[a]	13.58 ± 0.28[a]	2.27 ± 0.11	13.3 ± 0.2	0.03 ± 0.001	0.93 ± 0.04[a]	0.90 ± 0.04[a]	86 ± 1
ACO SUB (n = 16)	7.1 ± 0.1[b]	5.1 ± 0.1	0.24 ± 0.01[b]	10.2 ± 1.1	1.01 ± 0.20[b]	10.11 ± 0.18[b]	1.90 ± 0.10[b]	6.35 ± 0.25[b]	8.25 ± 0.20[b]	1.86 ± 0.09	11.5 ± 0.2	0.03 ± 0.002	0.64 ± 0.04[b]	0.60 ± 0.04[b]	82 ± 1
LAU TOP (n = 16)	6.7 ± 0.03	4.9 ± 0.04	0.25 ± 0.01	14.1 ± 2.1	1.02 ± 0.12	9.86 ± 0.27[a*]	2.73 ± 0.12[a*]	3.54 ± 0.20*	6.27 ± 0.23[a*]	3.58 ± 0.17[a*]	11.1 ± 0.3	0.03 ± 0.001	0.62 ± 0.03[a*]	0.60 ± 0.03[a*]	64 ± 2
LAU SUB (n = 16)	6.8 ± 0.01*	4.8 ± 0.01*	0.25 ± 0.01	22.4 ± 1.8	0.90 ± 0.15	7.17 ± 0.14[b*]	2.09 ± 0.10[b*]	2.99 ± 0.10*	5.08 ± 0.11[b*]	2.09 ± 0.11[b]	8.9 ± 0.2	0.03 ± 0.001	0.45 ± 0.02[b*]	0.42 ± 0.02[b*]	71 ± 1

EC – electrical conductivity; TN – total nitrogen; TOC – total organic carbon; HA+FA-C – carbon in humic and fulvic acids; HUM-C – carbon in humin; THC – total humic carbon; NHC – not humic carbon; WPCs, TPCs and HPCs – water-soluble, total and high-affinity phenolic compounds, respectively; THU – total level of humification. In columns: mean values followed by different letters are significantly different at $P = 0.05$ in the topsoil *vs.* subsoil comparison for each olive grove. The presence of an asterisk shows significant differences ($P = 0.05$) in the ACO *vs.* LAU comparison

The extracts were dissolved in methanol to obtain a final concentration of 1 mg/mL. Chromatographic analyses were conducted on an Agilent 1200 HPLC system (Agilent Technologies Inc., Palo Alto, USA); equipped with a binary pump, vacuum degasser, autosampler, thermostatic column compartment, and UV-Vis detector. A Luna C18-reversed phase column (5 µm particle size; 4.6 × 250.0 mm i.d.; Phenomenex) with a C-12 pre-column (4.0 mm × 3.0 mm; Phenomenex) was used for chromatographic separation at 25 °C and a flow rate of 0.7 mL/min. The UV detection of PCs was performed at 280 nm. The mobile phase consisted of 0.1% trifluoroacetic acid in water (*v*/*v*; mobile phase A) and acetonitrile (mobile phase B). The gradient program was run according to Luján et al. (2009) with a few modifications, namely 100% A 0–1 min; 84% A/16% B 1–10 min; 76% A/24% B 10–30 min; 64% A/36% B 30–40 min; 40% A/60% B 40–45 min, and 100% B 46–65 min, before re-equilibration to starting conditions. The injection volume of each sample was 20 µL.

Data were processed by Agilent ChemStation (A6.03.05) software (Santa Clara, USA). The PCs in olive leaf extracts were identified by comparison of their retention times with those of standard reference compounds and by means of their UV spectra. Quantitative analysis was performed using calibration curves of pure standard compounds, namely catechin, rutin, luteolin-7-*O*-glucoside, apigenin-7-*O*-rutinoside, luteolin-7-*O*-rutinoside, apigenin-7-*O*-glucoside, luteolin-3'-*O*-glucoside, diosmetin-7-*O*-glucoside, luteolin-4'-*O*-glucoside, luteolin and diosmetin as flavonoids, verbascoside as a cinnamic acid derivative, and oleuropein as a secoiridoid phenol. These PCs were chosen because they are the most representative in olive leaves (Abaza et al. 2015). The PCs in olive leaf and soil extracts were expressed in mg/g dry matter.

Statistical analysis. Statistics were conducted using R software program (The R Development Core Team 2015). Experimental data were compared using ANOVA. Statistical differences between mean values were determined using Tukey's post hoc honest significant difference test at $P < 0.05$. A correlation matrix (CM) based on the Pearson product-moment correlation coefficient was used to understand the relationships among the investigated parameters. Specifically, the raw dataset was base 10 log-transformed before creating the CM to reduce skewness and kurtosis toward a normal distribution (McDonald 2014).

https://doi.org/10.17221/165/2020-PSE

RESULTS AND DISCUSSION

Quantitative characterisation of soil phenolic compounds in core drill samples

Table 2 reports the chemical parameters, including the soil phenol contents, of the core drill samples (n = 64). The core soils showed a neutral-slightly alkaline pH_{H_2O} in both investigated stations, and, as well as the pedotypes, exhibited a significant potential acidity, as highlighted by the pH_{KCl} values, which was likely attributed to actual weathering of Al-silicates, particularly volcanic glass, although this was not very abundant. In both soils, total organic carbon (TOC), HA+FA-C, and HUM-C clearly decreased from the topsoil to the subsoil. The ACO topsoil showed the largest content of TOC regardless of which form was considered, with the exception of the non-humic fraction. This higher TOC content in the topsoil could be attributed to the presence of a permanent green cover and its root exudates. However, the spread of mowed grasses in place entails a very slow decomposition rate of such residues (Ghidey and Alberts 1993), which was reflected in the higher C:N ratio value in the ACO topsoil. Similar results were obtained by Castro

Table 3. Pearson's correlation coefficients among investigated properties in the soil core samples of Aconursi and Lauro olive groves

	pH_{H_2O}	pH_{KCl}	EC	WPCs	TPCs	HPCs	TOC	HA+FA-C	HUM-C	NHC	TN	C:N	Clay
Aconursi olive groves													
pH_{H_2O}	1												
pH_{KCl}	0.38*	1											
EC			1										
WPCs				1									
TPCs	−0.58**	0.35*	0.52**		1								
HPCs	−0.58**	0.35*	0.52**		1.00***	1							
TOC	−0.40*	0.40*	0.45*		0.62***	0.62***	1						
HA+FA-C	−0.41*	0.38*	0.42*		0.67***	0.67***	0.89***	1					
HUM-C							0.85***	0.55**	1				
NHC					0.43*	0.43*	0.41*	0.45*		1			
TN							0.42*	0.37*	0.38*		1		
C:N			0.48**		0.51**	0.52**	0.74***	0.67***	0.62***			1	
Clay	0.45**	−0.37*	−0.42*		−0.67***	−0.67***	−0.96***	−0.93***	−0.73***	−0.45**	−0.39*	−0.73***	1
Lauro olive groves													
pH_{H_2O}	1												
pH_{KCl}	0.52**	1											
EC			1										
WPCs		0.39*		1									
TPCs	−0.42*	0.42*			1								
HPCs	−0.43*	0.41*			0.99***	1							
TOC		0.50**			0.84***	0.84***	1						
HA+FA-C		0.44*			0.70***	0.70***	0.74***	1					
HUM-C							0.52**		1				
NHC	−0.36*				0.76***	0.77***	0.85***	0.57**		1			
TN	−0.37*				0.49**	0.50**				0.41*	1		
C:N		0.50**			0.60***	0.59***	0.86***	0.76***	0.49**	0.65***		1	
Clay				0.38*	−0.65***	−0.64***	−0.86***	−0.60***	−0.35*	−0.79***	−0.39*	−0.67***	1

EC – electrical conductivity; WPCs, TPCs and HPCs – water-soluble, total and high-affinity phenolic compounds, respectively; TOC – total organic carbon; TN – total nitrogen; HA+FA-C – carbon in humic and fulvic acids; HUM-C – carbon in humin; NHC – not humic carbon. *P < 0.05; **P < 0.01; ***P < 0.001; empty cell for not significant correlation

https://doi.org/10.17221/165/2020-PSE

et al. (2008) and Parras-Alcántara and Lozano-García (2014) in a Mediterranean olive grove under various soil-management techniques from tillage to organic farming.

The organic carbon content in the LAU soil was significantly lower than that detected in the ACO soil. The C:N ratio was quite low in the LAU subsoil compared with that in the topsoil and ACO samples. Such a decrease could be explained by the concurrent increase in clay level, which was in turn associated with a significant content of the well-decomposed organic fraction (HA+FA-C), which represented 29% of TOC (Table 2). Diekow et al. (2005) also achieved similar results.

In agreement with Rimmer and Abbott (2011) and Sadej et al. (2016), the concentration of TPCs was greater in surface samples than in subsurface samples, with a distribution almost similar to that of TOC where the largest TPC values were reached in the ACO topsoil and subsoil. In both olive groves, TPCs represented 5–6% of TOC on average. Furthermore, the distribution of WPCs did not appear to be related to either the TOC content or pedoclimatic conditions, reaching similar, very low amounts in the topsoil and subsoil. Consequently, the soil phenolic fraction with a high affinity for the soil body, namely HPCs, represented the largest percentage of PCs in the investigated soils, i.e., 95–97%, with a distribution similar to that of TPCs.

Several significant linear correlations were found among the chemical soil parameters, as highlighted by the Pearson CM in both olive groves (Table 3). In the ACO olive grove (Table 3), the strongest ($P < 0.001$) negative correlations were found among clay and TOC in humic and fulvic acids ($r = -0.96$ and $r = -0.95$, respectively). Other strong significant relationships existed between TOC and its humic fractions (HA+FA-C and HUM-C). The CM results confirmed the direct dependence of the TPC content on the amount of carbon, as well as the close relationship among TPCs and humic and fulvic acids, thereby supporting the polyphenols theory (Flaig 1988), which declares that phenols play a crucial role in humification pathways as important precursors in the formation of humic substances. The correlation coefficients confirmed that all the investigated chemical parameters affected the amount of WPCs. As attested by Kanerva et al. (2008), the concentration of TPCs in soil is positively correlated with the C:N ratio ($r = 0.51$; $P < 0.01$).

The CM outcomes for the LAU soils (Table 3) highlighted strong significant relationships similar to those found in the ACO soils. However, in the LAU soils, the content of TPCs, besides being strongly regulated by TOC, was also significantly ($P < 0.01$) correlated with the total nitrogen content. Our results agree with those found by Sądej et al. (2016). Furthermore, in the LAU soils, there was a slight ($P < 0.05$) correlation between the WPCs and the clay content ($r = 0.38$).

Table 4. The concentration of selected phenolic compounds (mg/g dry matter) in methanol leaf and soil extracts quantified by high-performance liquid chromatography equipped with an ultraviolet detector (HPLC-UV) analysis (mean value ± standard error)

Phenolic compound	Olive leaves, cv. Sessana	Aconursi		Lauro	
		topsoil	subsoil	topsoil	subsoil
Apigenin-7-*O*-glucoside	5.22 ± 0.07	0.04 ± 0.01	0.04 ± 0.01	0.04 ± 0.01	0.04 ± 0.01
Apigenin-7-*O*-rutinoside	4.41 ± 0.08	nd	nd	nd	nd
Catechin	2.76 ± 0.04	0.07 ± 0.02	0.08 ± 0.02	0.04 ± 0.01	0.04 ± 0.01
Diosmetin	2.91 ± 0.04	0.05 ± 0.01	0.04 ± 0.01	0.05 ± 0.01	0.03 ± 0.01
Diosmetin-7-*O*-glucoside	7.8 ± 0.1	nd	nd	nd	nd
Luteolin	10.5 ± 0.1	nd	nd	nd	nd
Luteolin-3'-*O*-glucoside	10.4 ± 0.1	nd	nd	nd	nd
Luteolin-4'-*O*-glucoside	12.1 ± 0.2	0.20 ± 0.10	0.30 ± 0.10	0.40 ± 0.10	0.40 ± 0.10
Luteolin-7-*O*-glucoside	4.10 ± 0.05	nd	nd	nd	nd
Luteolin-7-*O*-rutinoside	0.55 ± 0.01	nd	nd	nd	nd
Oleuropein	6.0 ± 0.1	nd	nd	nd	nd
Rutin	1.62 ± 0.03	0.07 ± 0.20[a]	0.03 ± 0.10[b]	0.70 ± 0.10	0.40 ± 0.10
Verbascoside	1.87 ± 0.03	0.80 ± 0.20	0.80 ± 0.20	1.00 ± 0.20	0.70 ± 0.20

In rows: mean values followed by different letters are significantly different at $P = 0.05$ in the topsoil *vs*. subsoil comparison for each olive grove. nd – not detectable

https://doi.org/10.17221/165/2020-PSE

Qualitative characterisation of selected phenolic compounds in leaf and core drill samples

Leaf characterisation. Table 4 reports the concentration of phenols found in cv. Sessana leaf extract. The secoiridoid oleuropein is reported as the most abundant PC in olive leaves, reaching 60–90 mg/g of their dry matter content (Kontogianni and Gerothanassis 2012). In our leaf extract, oleuropein represented only 8.5%. Such a low oleuropein content in the leaves was not surprising, as it can significantly vary among different olive cultivars (Blasi et al. 2016) and is affected by several other factors, such as geo-pedo-climatic features, agrotechniques, and sampling time (Papoti and Tsimidou 2009). All of these factors can considerably influence the abundance and distribution of phenols in olive leaves to the extent that flavonoids can represent the major phenol constituents in olive leaves (Goulas et al. 2010). Our data highlighted that flavonoids account for 88.8% of the investigated PCs in leaf extracts. The flavone luteolin and its 3'-*O*-glucoside and 4'-*O*-glucoside, whose contents were estimated to equal 10.5, 10.4, and 12.1 mg/g dry matter, respectively, were the most abundant flavonoids.

Core drill characterisation. The concentrations of selected PCs in the soil extracts are reported in Table 4. In such extracts, more than half of the identified leaf PCs were absent. Notably, oleuropein, which is the most characteristic olive leaf phenol, was not found in the soils. The differences between the concentrations in the topsoil and subsoil were not statistically significant ($P > 0.05$), with the exception of rutin in the ACO olive grove. The abundance of the detected PCs decreased in the order of verbascoside > luteolin-4'-*O*-glucoside >> rutin, catechin, diosmetin, and apigenin-7-*O*-glucoside in ACO and verbascoside > rutin > luteolin-4'-*O*-glucoside >> diosmetin, catechin, and apigenin-7-*O*-glucoside in LAU. The concentration of rutin in the LAU soil was much higher than that in the ACO soil, and verbascoside and rutin tended to accumulate in the LAU topsoil.

The persistence of PCs in soils appears to be dependent on various biotic and abiotic environmental conditions, such as the presence of microbes, their susceptibility to photochemical oxidation, and adsorption to soil particles and organic matter, and the subject is still debated (Weston and Mathesius 2003). Some works (Sosa et al. 2010 and references therein) have reported the discontinuous persistence of flavonoids in soil, and according to Barto and Cipollini (2009), flavonoid glycosides are not detected in bulk soils. Most flavonoid glycosides are rapidly hydrolysed by microorganisms and plant exoenzymes in soil, and their persistence can be less than 72 h (Hassan and Mathesius 2012). All the above-cited PCs not found in soil contain easily accessible sugars that can be hydrolysed/metabolised. However, in contrast to this observation, luteolin, which was not found in the soil, has no sugar, whereas the glycosides rutin, verbascoside, luteolin-4'-*O*-glucoside, and apigenin-7-*O*-glucoside showed persistence in soil.

The persistence of catechin, which is the main constituent of condensed tannins, is due to its recalcitrant nature and resistance to microbial attack and also because it is toxic to plants, animals, and microorganisms (Arunachalam et al. 2003). According to some authors, catechin toxicity justifies its stability in the soil environment. Contrastingly, other authors claim that it is unstable (Cesco et al. 2012). Similarly, the allelophatic effect of rutin (Golisz et al. 2007) and verbascoside (Senatore et al. 2007) could explain their persistence. Specifically, their higher stability in the LAU soils than in the ACO soils could be explained by the significant clay content in the former (Table 2). As stated by Dalton (1999), the presence of functional groups that promote plant toxicity also boosts molecular sorption on soil clay particles.

In addition, the lack of oleuropein could be explained by taking into account the fact that this secoiridoid is classified as a hydrophilic phenol; thus, it is highly soluble and mobile in the soil environment (Panizzi et al. 1960). The significantly different phenolic pattern ascertained between the ACO and LAU core samples could be ascribed to the presence of a substantial grass cover in the ACO topsoil. The grass cover could act in two possibly synergistic ways, namely (*i*) favoring leaf decay above the soil surface with concomitant biotic and abiotic hydrolysis and oxidation of cell lysate and (*ii*) sustaining the activity of rhizosphere biomass, which is able to metabolise the PCs. This agrees with the active humification processes in such soil (Table 2). Furthermore, in the ACO soils, the coarser texture could have enhanced the lisciviation process with removal of some compounds in soil solution.

As reported in the introduction, there are still few data on soil phenols concentration, distribution, and behavior in both space and time, notwithstanding they perform several roles, such as control in soil organic matter dynamics and nutrient cycling, directly interfering with ion transport, and energy metabolism. Specifically, flavonoids are considered indicative compounds in

https://doi.org/10.17221/165/2020-PSE

plant-microorganisms symbiosis, representing a tool for nitrogen fixation in infertile soils (Palma-Tenago et al. 2017). Our results provide preliminary information on the distribution of phenols in olive grove soils, thus contributing at the understanding of factors and processes which drive the behavior and the fate of phenols within the soil-plant system. Furthermore, our outcomes could be useful to understand the bioavailability of such compounds in future studies on allelopathic interferences in field situations.

Acknowledgments. A special thanks to prof. Andrea Buondonno, a brilliant soil scientist who passed away one year ago, that supported all phases of this work.

REFERENCES

Abaza L., Taamalli A., Nsir H., Zarrouk M. (2015): Olive tree (*Olea europeae* L.) leaves: importance and advances in the analysis of phenolic compounds. Antioxidants, 4: 682–698.

Arunachalam M., Mohan Raj M., Mohan N., Mahadevan A. (2003): Biodegradation of catechin. Proceedings of the Indian National Science Academy, B69: 353–370.

Barto E.K., Cipollini D. (2009): Half-lives and field soil concentrations of *Alliaria petiolata* secondary metabolites. Chemosphere, 76: 71–75.

Blakemore L.C., Searle P.L., Daly B.K. (1987): Methods for Chemical Analysis of Soils. Scientific Report, 80. Lower Hutt, New Zealand Soil Bureau.

Blasi F., Urbani E., Simonetti M.S., Chiesi C., Cossignani L. (2016): Seasonal variations in antioxidant compounds of *Olea europaea* leaves collected from different Italian cultivars. Journal of Applied Botany and Food Quality, 89: 202–207.

Box J.D. (1983): Investigation of the Folin-Ciocalteau phenol reagent for the determination of polyphenolic substances in natural waters. Water Research, 17: 511–525.

Buondonno A., Capra G.F., Coppola E., Dazzi C., Grilli E., Odierna P., Rubino M., Vacca S. (2014): Aspects of soil phenolic matter (SPM): an explorative investigation in agricultural, agroforestry, and wood ecosystems. Geoderma, 213: 235–244.

Castro J., Fernández-Ondoño E., Rodríguez C., Lallena A.M., Sierra M., Aguilar J. (2008): Effects of different olive-grove management systems on the organic carbon and nitrogen content of the soil in Jaén (Spain). Soil and Tillage Research, 98: 56–67.

Regional Agrometeorological Centre (2019): Available at: http://www.agricoltura.regione.campania.it/meteo/agrometeo.htm (accessed 13 June 2019)

Cesco S., Mimmo T., Tonon G., Tomasi N., Pinton R., Terzano R., Neumann G., Weisskopf L., Renella G., Landi L., Nannipieri P. (2012): Plant-borne flavonoids released into the rhizosphere: impact on soil bio-activities related to plant nutrition. A review. Biology and Fertility of Soils, 48: 123–149.

Chomel M., Guittonny-Larchevêque M., Fernandez C., Gallet C., DesRochers A., Paré D., Jackson B.J., Baldy V. (2016): Plant secondary metabolites: a key driver of litter decomposition and soil nutrient cycling. Journal of Ecology, 104: 1527–1541.

Costantini E.A.C., Urbano F., L'Abate G. (2004): Soil regions of Italy. Available at: www.soilmaps.it

Dalton B.R. (1999): The occurrence and behavior of plant phenolic acids in soil environments and their potential involvement in allelochemical interference interactions: methodological limitations in establishing conclusive proof of allelopathy. In: Inderjit, Dakshini K.M.M., Foy C.L. (eds.): Principles and Practices in Plant Ecology. Allelochemical Interactions. Boca Raton, CRC Press, 57–74. ISBN 9780849321160

De Carvalho P.L.N., de Oliveira Silva E., Aparecida Chagas-Paula D., Hortolan Luiz J.H., Ikegaki M. (2016): Importance and implications of the production of phenolic secondary metabolites by endophytic fungi: a mini-review. Mini-Reviews in Medicinal Chemistry, 16: 259–271.

Dell'Abate M.T., Benedetti A., Trinchera A., Dazzi C. (2002): Humic substances along the profile of two Typic Haploxerert. Geoderma, 107: 281–296.

Diekow J., Mielniczuk J., Knicker H., Bayer C., Dick D.P., Kögel-Knabner I. (2005): Soil C and N stocks as affected by cropping systems and nitrogen fertilisation in a southern Brazil Acrisol managed under no-tillage for 17 years. Soil and Tillage Research, 81: 87–95.

Flaig W.J.A. (1988): Generation of model chemical precursor. In: Frimmel F.H., Christman R.F. (eds.): Humic Substances and Their Role in the Environment. Chichester, J. Wiley and Sons Ltd., 75–78. ISBN 0-471-91871-2

Gallet C., Pellissier F. (1997): Phenolic compounds in natural solutions of a coniferous forest. Journal of Chemical Ecology, 23: 2401–2412.

Ghidey F., Alberts E.E. (1993): Residue type and placement effects on decomposition: field study and model evaluation. Transactions of the American Society of Agricultural Engineers, 36: 1611–1617.

Golisz A., Lata B., Gawronski S.W., Fujii Y. (2007): Specific and total activities of the allelochemicals identified in buckwheat. Weed Biology and Management, 7: 164–171.

Goulas V., Papoti V.T., Exarchou V., Tsimidou M.Z., Gerothanassis I.P. (2010): Contribution of flavonoids to the overall radical scavenging activity of olive (*Olea europaea* L.) leaf polar extracts. Journal of Agricultural and Food Chemistry, 58: 3303–3308.

Hassan S., Mathesius U. (2012): The role of flavonoids in root-rhizosphere signaling: opportunities and challenges for improving plant-microbe interactions. Journal of Experimental Botany, 63: 3429–3444.

Hättenschwiler S., Vitousek P.M. (2000): The role of polyphenols in terrestrial ecosystem nutrient cycling. Tree, 15: 238–243.

IUSS Working Group WRB (2015): World Reference Base for Soil Resources 2014, Update 2015 International Soil Classification System for Naming Soils and Creating Legends for Soil Maps. World Soil Resources Reports No. 106. Rome, Food and Agriculture Organization.

Kaiser K., Guggenberge G., Haumaier L. (2004): Changes in dissolved lignin-derived phenols, neutral sugars, uronic acids, and amino sugars with depth in forested Haplic Arenosols and Rendzic Leptosols. Biogeochemistry, 70: 135–151.

https://doi.org/10.17221/165/2020-PSE

Kanerva S., Kitunen V., Loponen J., Smolander A. (2008): Phenolic compounds and terpenes in soil organic horizon layers under silver birch, Norway spruce and Scots pine. Biology and Fertility of Soils, 44: 547–556.

Kontogianni V.G., Gerothanassis I.P. (2012): Phenolic compounds and antioxidant activity of olive leaf extracts. Natural Product Research, 26: 186–189.

Li Z.H., Wang Q., Ruan X., Pan C.D., Jiang D.A. (2010): Phenolics and plant allelopathy. Molecules, 15: 8933–8952.

Lowe L.E. (1993): Water-soluble phenolic materials. In: Carter M.R., Gregorich E.G. (eds.): Soil Sampling and Methods of Analysis. Boca Raton, Lewis Publishers, 409–412. ISBN-13: 978-0-8493-3586-0

Luján R.J., Capote F.P., de Castro M.D. (2009): Temporal metabolomic analysis of o-glucoside phenolic compounds and their aglycone forms in olive tree and derived materials. Phytochemical Analysis, 20: 221–230.

Mahugo Santana C., Sosa Ferrera Z., Torres Padrón M.E., Santana Rodríguez J.J. (2009): Methodologies for the extraction of phenolic compounds from environmental samples: new approaches. Molecules, 14: 298–320.

Makoi J.H.J.R., Ndakidemi P.A. (2007): Biological, ecological and agronomic significance of plant phenolic compounds in rhizosphere of the symbiotic legumes. African Journal of Biotechnology, 6: 1358–1368.

Malá J., Cvikrová M., Hrubcová M., Máchová P. (2013): Influence of vegetation on phenolic acid contents in soil. Journal of Forensic Sciences, 59: 288–294.

Massaccesi L., De Feudis M., Agnelli A.E., Nasini L., Regni L., D'Ascoli R., Castaldi S., Proietti P., Agnelli A. (2018): Organic carbon pools and storage in the soil of olive groves of different age. European Journal of Soil Science, 69: 843–855.

McDonald J.H. (2014): Handbook of Biological Statistics. 3rd Eition. Baltimore, Sparky House Publishing, 299. ISBN 9783540747062

Min K.J., Freeman C., Kang H.J., Choi S.-U. (2015): The regulation by phenolic compounds of soil organic matter dynamics under a changing environment. BioMed Research International, 2015, ID 825098.

MiPAF – Granco A (ed.) (2000): Chemical Methods of Soil Analysis. Milan, Italian Ministry of Agriculture and Forestry Politics. (In Italian)

Muscolo A., Sidari M., Texeira da Silva J.A. (2013): Biological effects of water-soluble soil phenol and soil humic extracts on plant systems. Acta Physiologiae Plantarum, 35: 309–320.

Northup R.R., Dahlgren R.A., McColl J.G. (1998): Polyphenols as regulators of plant-litter-soil interactions in Northern California's Pygmy forest: a positive feedback? Biogeochemistry, 42: 189–220.

Palma-Tenango M., Soto-Hernández M., Aguirre-Hernández E. (2017): Flavonoids in agriculture. In: Goncalo C.J. (ed.): Flavonoids – From Biosynthesis to Human Health. London, IntechOpen, 189–201. ISBN: 978-953-51-3424-4

Panizzi L.M., Scarpati M.L., Oriente E.G. (1960): Structure of oleuropein, a bitter glucoside with hypotensive activity from olive tree. Italian Chemical Gazette, 90: 1449–1485.

Papoti V.T., Tsimidou M.Z. (2009): Impact of sampling parameters on the radical scavenging potential of olive (*Olea europaea* L.) leaves. Journal of Agricultural and Food Chemistry, 57: 3470–3477.

Parras-Alcántara L., Lozano-García B. (2014): Conventional tillage versus organic farming in relation to soil organic carbon stock in olive groves in Mediterranenan rangelands (southern Spain). Solid Earth, 5: 299–311.

Rimmer D.L., Abbott G.D. (2011): Phenolic compounds in NaOH extracts of UK soils and their contribution to antioxidant capacity. European Journal of Soil Science, 62: 285–294.

Rubino M., Benedetti A., Coppola E., Dell'Abate M.T., Buondonno A. (2008): A new oxidation method for determination of soil extracted an humic substances. In: Dazzi C., Costantini E. (eds.): The Soils of Tomorrow: Soils Changing in a Changing World. Advances in Geoecology, 39. Reiskirchen, Catena Verlag, 719–728. ISBN: 978 3 923381 56 2

Sądej W., Żołnowski A.C., Marczuk O. (2016): Content of phenolic compounds in soils originating from two long-term fertilization experiments. Archives of Environmental Protection, 42: 104–113.

Schmidt M.W.I., Torn M.S., Abiven S., Dittmar T., Guggenberger G., Janssens I.A., Kleber M., Kögel-Knabner I., Lehmann J., Manning D.A.C., Nannipieri P., Rasse D.P., Weiner S., Trumbore S.E. (2011): Persistence of soil organic matter as an ecosystem property. Nature, 478: 49–56.

Schoeneberger P.J., Wysocki D.A., Benham E.C. (2012): Field Book for Describing and Sampling Soils. Version 3.0. Lincoln, Natural Resources Conservation Service, National Soil Survey Center.

Senatore F., Rigano D., Formisano C., Grassia A., Basile A., Sorbo S. (2007): Phytogrowth-inhibitory and antibacterial activity of *Verbascum sinuatum*. Fitoterapia, 78: 244–247.

Geological Survey of Italy (1966): Geological Map of Italy 1:100.000 -sheet 171 GAETA

Sinsabaugh R.L. (2010): Phenol oxidase, peroxidase and organic matter dynamics of soil. Soil Biology and Biochemistry, 42: 391–404.

Soil Survey Laboratory Staff (1996): Soil Survey Laboratory Methods Manual. Soil Survey Investigations Rep. 42 V. 3.0. USDA-NRCS.U.S. Washington, US Government Printing Office.

Soil Survey Staff (2014): Soil Survey Field and Laboratory Methods Manual. Soil Survey Investigations Report No. 51, Version 2.0. In: Burt R., Soil Survey Staff (eds.): U.S. Department of Agriculture, Natural Resources Conservation Service.

Sosa T., Valares C., Alías J.C., Lobón N.C. (2010): Persistence of flavonoids in *Cistus ladanifer* soils. Plant and Soil, 337: 51–63.

Sugiyama A., Yazaki K. (2014): Flavonoids in plant rhizospheres: secretion, fate and their effects on biological communication. Plant Biotechnology, 31: 431–443.

The R Development Core Team (2015): R: A Language and Environment for Statistical Computing. Vienna, R Foundation for Statistical Computing. Available at: http://www.R-project.org/ (accessed 13 October 2015)

Weston L.A., Mathesius U. (2013): Flavonoids: their structure, biosynthesis and role in the rhizosphere, including allelopathy. Journal of Chemical Ecology, 39: 283–297.

Received: March 31, 2020
Accepted: April 30, 2020
Published online: May 12, 2020