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Short-term effects of olive oil mill wastewater application on soil water repellency

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Abstract

Land spreading of olive mill wastewater (OMW) allows a cheap and environmentally sound effluent disposal. However, this practice requires suitable application protocols, in order to avoid negative effects on soil hydrological properties, such as the increase of soil water repellency (SWR). The effects of OMW land spreading on SWR, mainly evaluated in the long term, have been rarely measured few days or weeks after land spreading. To this purpose, this study has evaluated the short-term effects of OMW land spreading on SWR of olive groves (with silt, loam or silty clay loam soil texture) using the Water Drop Penetration Test (WDPT) at laboratory scale. SWR significantly ($p < 0.001$) varied with the soil texture and depth (surface layer or 10-cm depth) as well as the treatment (land spreading of OMW or fresh water, FW) and time elapsed from land application. More specifically, SWR was significantly higher ($p < 0.001$) in the topsoil than the sub-surface layer. Compared to the soils irrigated with FW, a 2-fold WDPT (thus a slightly higher SWR) was found after OMW application in both soil layers, regardless of the texture. However, this weak SWR disappears just after two weeks from land spreading, and the hydrophobicity of the soils treated with OMW and FW becomes very similar. Moreover, the topsoil and sub-surface layer showed the same SWR after four weeks. The high coefficients of determination ($r^2 > 0.86$) in the linear regressions between WDPT and OM content of soils proved the clear influence of the organic compounds on SWR, which decreases with OM, as expected. Overall, OMW land spreading has not significantly changed SWR, at least under the limited hydraulic and organic loads adopted in this study, and less noticeably on loam or silty clay loam soil compared to sandy loam texture. However,

50 this practice is more viable in spring or autumn, since in these seasons the risk of groundwater
51 contamination is particularly reduced.

52

53 **Keywords:** Water Drop Penetration Test; soil hydrophobicity; OMW land spreading; soil organic
54 matter; wastewater management.

55

56 1. Introduction

57

58 Olive oil production is a primary agro-industrial activity in many Mediterranean countries (Mateo et
59 al., 2015; Fountoulakis et al., 2008). Olive processing produces large amounts of residues: a very
60 wet cake, the so-called "olive pomace", and a liquid stream, called "olive mill wastewater" (OMW).
61 The latter is generated during the different stages of oil production and by the water used for
62 cleaning purposes (Moreno et al., 2017). OMW has a dark colour, characteristic odour, low pH, and
63 contains high concentrations of fats, oils and greases (FOGs), organic matter (OM), suspended
64 solids and pollutant compounds, such as polyphenols. The presence of polyphenols as well as short
65 and long-chain fatty acids of FOGs contribute to the phytotoxic and antimicrobial effects of OMW
66 (Saadi et al., 2007). Because of these characteristics, OMW management poses serious
67 environmental risks to water, soil and air. As a matter of fact, the uncontrolled disposal of the oil
68 industry effluents may cause water body pollution, soil degradation and odour emissions (Dermeche
69 et al., 2013; Chaari et al., 2015).

70 The techniques applied for OMW depuration (physico-chemical or biological treatments) are
71 complex and expensive (Dourou et al., 2016; Calabrò et al., 2018). In the last decades, novel bio-
72 technologies have been proposed and tested for the OMW treatment and valorisation, such as the
73 production of phenolic compounds (e.g., Tsioulpas et al., 2002; Aggelis et al., 2003), citric acid
74 (Papanikolaou et al., 2008), single cell oil (Bellou et al., 2014), bio-ethanol (Sarris et al., 2014) and
75 added-value metabolites (Sarris et al., 2017). These bio-technologies techniques seem to be
76 promising, but not yet consolidated in the common practice.

77 Therefore, these management options may be economically unsustainable for the smallest oil mills
78 (Calabrò et al., 2018; Diamantis et al., 2013b). A viable solution is OMW land spreading, which
79 consists of the controlled application of the oil industry effluents to cultivated soil. Through soil
80 application, OMW can be used as a cheap soil conditioner and/or fertilizer (Barbera et al., 2014).
81 Moreover, OMW is an additional water resource for the Mediterranean agricultural areas, affected
82 by a chronic water and OM scarcity (Chaari et al., 2015). By this practice, the treatment cost related
83 to the large volume of OMW produced by oil mills can be reduced.

84 Despite these environmental and economic benefits, OMW land spreading must be practised with
 85 caution. The agronomic reuse of OMW without following suitable protocols for soil application can
 86 degrade soil characteristics (S'habou et al., 2009). The effects of OMW land spreading have been
 87 largely studied, particularly in the long terms (Zema et al., 2019). On this regard, Mekki et al.
 88 (2006) and Barbera et al. (2013) have issued two interesting reviews discussing the effects of OMW
 89 land spreading on soils and crops of different characteristics. For instance, it has been demonstrated
 90 that OMW land spreading induces beneficial effects on the physico-chemical (organic matter and
 91 nutrients) and microbiological (arbuscular mycorrhizal fungi) (e.g., Caruso et al., 2018;
 92 Chatzistathis and Koutsos, 2017) properties of the treated soils; potential phytotoxicity to some
 93 crops has been reported in some studies (e.g., Saadi et al., 2007).

94 However, literature data about OMW effects on soil properties are not unanimous and, in some
 95 cases, contradictory, since the effluents, whose qualitative variability (due to the high variability of
 96 pH, and the wide range of concentrations of total solids as well as of organic matter and
 97 polyphenols) is large, are applied at several hydraulic rates and over soils of different characteristics
 98 (Chartzoulakis et al., 2010).

99 As regards the hydrological characteristics of the soils receiving OMW, much caution should be
 100 paid to the possible reduction in the water infiltration after application. In the Mediterranean areas,
 101 where the infiltration-excess mechanism dominates the soil hydrological response (Lucas-Borja et
 102 al., 2018), a reduced infiltration capacity could make these areas particularly prone to runoff and
 103 soil erosion risks (Fortugno et al., 2017). Moreover, since OMW also contains residual oil (1.2-1.4
 104 kg per 100 kg of treated olives, Abegunrin et al., 2016; Servili et al., 2004), the wax-like substances
 105 of OMW can form a coating on soil particles (Bisdorf et al., 1993). Therefore, under the
 106 Mediterranean conditions, the soils irrigated with OMW could become hydrophobic (Tarchitzky et
 107 al., 2007; Travis et al., 2008). Soil hydrophobicity, also known as soil water repellency (hereinafter
 108 "SWR") (Abegunrin et al., 2016; DeBano, 1969; Doerr et al., 2000;), is the situation whereby the
 109 soil does not wet when water is spontaneously applied (Wallach and Graber, 2007). SWR presence
 110 has been documented in various regions, climates, soils and land uses (Doerr et al., 2000; Ritsema
 111 and Dekker, 2003). This effect induces degradation of soil hydrological properties, such as
 112 reduction of water infiltration. SWR is influenced by several soil properties and conditions (e.g.
 113 OM content, texture, pH, water content) (Doerr et al., 2000). For instance, soil OM strongly affects
 114 SWR (Graber et al., 2006; Serres, 1992; Wallach et al., 2005). The presence of organic compounds
 115 derived from living or decomposing plants or microorganisms coat soil particle surfaces and
 116 aggregates, making it repellent to the water infiltration (Abegunrin et al., 2016). SWR is found on
 117 coarse textured soils (where it is more pronounced), but is also common in fine textured soils,

118 where a high level of hydrophobicity is possible (Doerr et al., 2000; Doerr et al., 2006). Moreover,
119 SWR usually occurs when soils dry out to below a critical soil water content (Dekker et al., 2001;
120 Wallis and Horne, 1992), which is common in the dry seasons of the Mediterranean climate.
121 Therefore, the OMW land spreading on dry soils may generate or even aggravate SWR with the
122 subsequent worsening of hydrological response also in the dry seasons.
123 Several studies have evaluated the effects of wastewater on SWR on different soil types (e.g.,
124 Debano, 2000; Wallach et al., 2005). The experiences dealing with OMW land spreading are less
125 numerous, and have been carried out mainly on the long term (e.g. Mahmoud et al. 2010; Peikert et
126 al. 2015). The evaluation of the effects of OMW on the physical properties of soil (thus including
127 SWR) performed some weeks or months after application are equally important, since many
128 adverse effects may be present only in this period (Zema et al., 2019). Therefore, there is the need
129 of studies evaluating how and by what extent SWR of the Mediterranean soils may change
130 immediately or few weeks after receiving OMW.
131 To fill this gap, this study evaluates the short-term effects (at 2, 7, 14 and 21 days) of OMW
132 application on SWR of sandy loam, silt loam and silty clay loam soils at different depths (surface
133 and at a depth of 10 cm) at laboratory scale. We hypothesised that the high contents of OM and
134 hydrophobic substances in OMW may noticeably alter SWR, depending on the soil depth and the
135 time elapsed since OMW application. Overall, these short-term effects after OMW land spreading
136 are expected to be negative, leading to the SWR increase, particularly at the soil surface.

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138 **2. Materials and methods**

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140 *2.1. Study areas*

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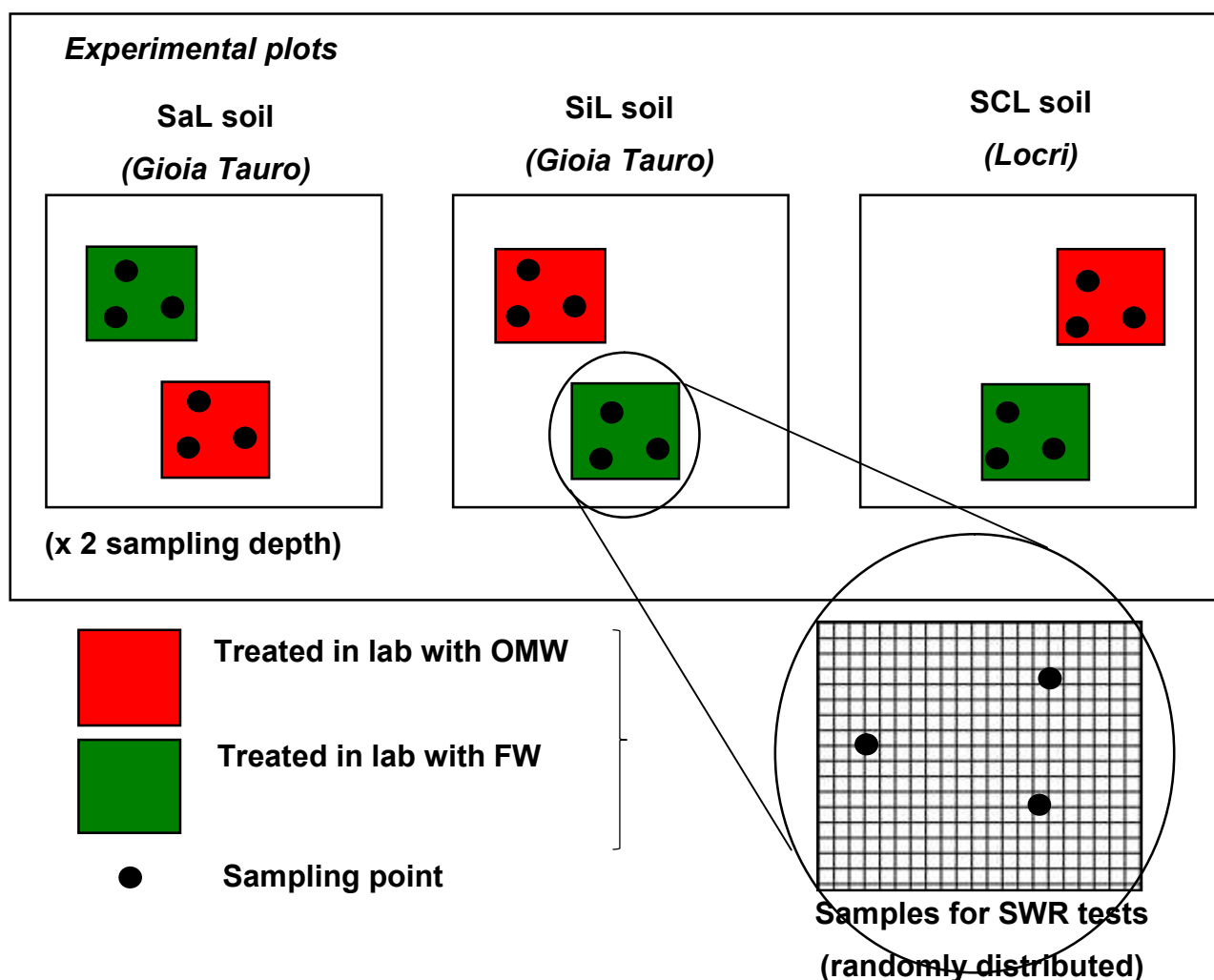
142 The investigation was carried out in olive groves (*Olea europea*) of two farms in Calabria, Southern
143 Italy (Figure 1a), of which one is located in Locri and the other in Gioia Tauro. The climate of both
144 farms is typically semi-arid hot-summer Mediterranean climate, Csa class, according to Koppen
145 (1918). The annual rainfall and minimum/maximum temperatures are on average 1300-1400 mm
146 and 11-28 °C, respectively (historical observations of 1923-2017 of Environmental Protection
147 Agency of Calabria Region, ARPACAL).

148 The olive grove in Locri (38.2671° N, 16.1872° E, mean altitude of 114 m above mean sea level) is
149 planted with trees of cultivar *Geracese* (about 10-12 years old) at 6 m x 6 m spacing. The olive
150 grove in Gioia Tauro (38.4136° N, 15.9351° E, mean altitude of 10 m a.s.l.), is planted with trees of

151 cultivar *Sinopolese* (20-year old) planted at 5 m x 4 m. Both olive groves are usually subject to
152 mechanical tillage twice a year and weed removal, using disc-ploughs and harrows, respectively.
153 In the olive groves, three plots with as many soil types were identified, in order to catch the SWR
154 variability among different textures. One plot, covering an area of 250 m² (42 m x 6 m), was in
155 Locri. The two other plots, of 2 x 360 m² (24 m x 15 m) were set in the olive grove of Gioia Tauro.
156 In each plot, two areas were chosen; three soil samples per area were collected for the subsequent
157 treatments and SWR tests in laboratory (Figure 1b). More details about the characteristics of the
158 experimental sites can be found in the works of Andiloro et al. (2007) and Bombino et al. (2019).



(a)



(b)

Figure 1 - Geographic location of the investigated olive groves (a) and experimental design for the SWR tests (b).

According to the USDA/FAO Soil classification (Soil Survey Division Staff, 1993), the soils were characterised on 30 to 45 samples per plot (collected at a depth of 10 cm below ground), as:

(i) sandy loam (on the average 70% w/w of sand, 24% of silt and 6% of clay and 3% of skeleton) in one plot of Gioia Tauro;

(ii) silt loam (19% of sand, 71% of silt and 10% of clay and a lack of skeleton) in the second plot of Gioia Tauro;

(iii) silty clay loam (2% of sand, 70% of silt and 28% of clay with 3% of skeleton) in the plot of Locri.

Hereinafter the three soils will be indicated as SaL, SiL and SCL, respectively.

2.2. Experimental design

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2.2.1. Soil sampling

Eighteen soil samples (3 soils x 2 treatments x 3 replicates) were extracted in late May from the three plots using a steel ring (0.32 m in diameter and 0.26 m in height). Before sampling, rocks and weeds were removed over the soil surface. Then, the ring was inserted into the soil by pressing. The soil sample was carefully extracted and transported to the laboratory, where the sample gently put in the same day in a 18.5-litre pot (diameter of 0.30 m and depth of 0.26 cm) with an upper surface area of 0.07 m². The pots were stored in a climatic chamber at 20 °C until the dates of land spreading and SWR measurement.

The day after collection, the samples were irrigated with 0.71 litres (about 100 m³ ha⁻¹) of OMW (treated soil) or fresh water (hereinafter, FW). The latter treatment, in which groundwater was used, was considered as control. In this study, the hydraulic load of OMW supply was within the same order of the maximum limit permitted by the Italian law n. 574/96, equal to 80 m³ ha⁻¹ per year.

2.2.2. SWR measurement

SWR was measured in each pot containing the soil samples at two, seven, fourteen and twenty-one days (henceforth indicated as T₂, T₇, T₁₄ and T₂₁, respectively) after land spreading of OMW or FW. According to Letey (1969), the water drop penetration time (WDPT), according to the methods proposed by Van't Woudt in 1959 and commonly accepted in literature (Letey et al., 2000; Buczko and Bens, 2006; Tarchitzky et al., 2007), was used to evaluate SWR. In more detail, two soil samples were gently collected at the measurement dates from the surface layer (SL) and, on the same vertical line, at a depth of 10 cm (sub-surface layer, SSL) of every pot, caring to sampling the soil from a different area of the pot surface at each date. After the measurement, the sampled soils were restored in the pot.

The samples were sieved at 2-mm sieve. The material of the samples was filled into circular dishes of 10-cm diameter and the soil surface was manually smoothed. Field-moist samples were used instead of oven-dried soil, in order to measure more realistic values of SWR instead of the “potential” repellency (Buczko and Bens, 2006). Since the samples were collected at the same date from soils with practically the same characteristics, the variability of the water content (10.4 ± 1.1 for SaL, 19.1 ± 2.4 for SiL and 18.3 ± 1.9% for SCL soil) was very low. Moreover, since all the samples were stored under the same conditions, significant changes in their water content were not expected.

211 A total of 10 - 15 drops of distilled water were applied to the surface of the soil samples through a
212 medical pipette (water volume of one droplet: $58 \pm 5 \mu\text{l}$) and the WDPT was recorded (Mahmoud et
213 al., 2010). According to Bisdom et al. (1993), the SWR was classified as follows:

214

215 - wettable or non water-repellent soil (WDPT < 5 seconds)

216 - slightly water-repellent soil (WDPT = 5 - 60 s)

217 - strongly water-repellent soil (WDPT = 60 - 600 s)

218 - severely water-repellent soil (600 - 3600 s)

219 - extremely water-repellent soil (WDPT > 3600 s).

220

221 Overall, the experimental design consisted of two treatments (OMW vs FW) x two soil depths (SL
222 vs SSL) x four dates (T_2 vs T_7 vs T_{14} vs T_{21}) x three soils (SaL vs SiL vs SCL) x three replicates
223 (spatially independent and randomly established) for a total of 144 tests.

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225 2.2.3. Wastewater and soil characterization

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227 OMW was collected from a local olive oil processing plant using a continuous 3-phase extraction
228 system. The OMW samples were stored in an open concrete tank for about 30 days prior to land
229 application (as usually done to face off the time variability of OMW production).

230 The main chemical-physical properties of OMW and FW were determined in triplicate immediately
231 before the soil watering (Table 1). The Italian standards (APAT, 2003), which refer to the common
232 international methods (APHA-AWWA-EF, 1998; ASTM, 1981; EPA, 1974), were adopted for the
233 analyses. The polyphenol concentration of OMW was determined by using Folin-Ciocalteu
234 method (Folin and Ciocalteu, 1927).

235

236 Table 1 - Main chemical-physical properties of OMW and FW used in the SWR tests (n = 3
 237 samples).

238

Parameter	Applied water type	
	OMW	FW
<i>pH (-)</i>	4.14 ± 0.91 a	6.31 ± 0.69 b
<i>Total suspended solids (mg l⁻¹)</i>	5280 ± 190 a	14.0 ± 3.78 b
<i>Settleable solids (mg l⁻¹)</i>	698 ± 105 b	0 a
<i>Electrical conductivity (mS cm⁻¹)</i>	1.58 ± 0.70 a	1.42 ± 0.51 a
<i>COD (g l⁻¹)</i>	12.7 ± 3.68 b	0 a
<i>Total nitrogen (mg l⁻¹)</i>	89.0 ± 4.45 a	1.10 ± 0.10 a
<i>Polyphenols (g l⁻¹)</i>	0.65 ± 0.36 b	0 a

239 Notes: OMW = olive oil mill wastewater; FW = clean water; COD = chemical oxygen demand; different lowercase
 240 letters indicate significant differences after t-test at p-level < 0.05.

241

242 The following properties over the fraction finer than 2 mm (after sample air-drying and sieving)
 243 were determined on three composite samples of surface and sub-surface layers in the three soils
 244 (Table 2): (i) pH, by portable electrochemical instrument Hach Lange HQ30d (Hach Company,
 245 Loveland, Colorado, USA); (ii) OM content, by Walkey and Black method (Walkey and Black,
 246 1934); (iii) total carbon and nitrogen, by elemental analyzer LECO CN628 (LECO Corporation,
 247 Michigan, USA), carried out on samples crushed to pass through a 500-µm sieve.

248

249 Table 2 - Main physico-chemical properties of the three soils before the SWR tests (n = 3).

250

Parameter	Soil type		
	SaL	SiL	SCL
<i>pH (-)</i>	5.80 ± 0.09 a	5.50 ± 0.08 a	8.20 ± 0.16 b
<i>OM content (%)</i>	2.80 ± 0.45 ab	3.80 ± 0.44 b	1.94 ± 0.03 a
<i>Total carbon (%)</i>	1.60 ± 0.10 ab	2.20 ± 0.11 b	1.13 ± 0.02 a
<i>Total nitrogen (%)</i>	0.90 ± 0.03 b	2.60 ± 0.13 c	0.19 ± 0.01 a

251 Notes: SaL = sandy loam; SiL = silt loam; SCL = silty clay loam; OM = Organic Matter; different lowercase letters
 252 indicate significant differences after t-test at p-level < 0.05.

253

2.3. Statistical analysis

The statistical analysis was carried out using the three-way ANOVA. Treatment (OMW or FW), soil depth (SL or SSL) and time (T₂, T₇, T₁₅ or T₂₁) were chosen as factors, while SWR was considered as the response variable. The pairwise comparison by Tukey's test (at $p < 0.001$) was also used to evaluate the statistical significance of the differences in SWR among factors. In order to satisfy the assumptions of the statistical tests (equality of variance and normal distribution), the data were subjected to normality test or were square root-transformed whenever necessary. Moreover, a regression analysis between WDPT and the OM content of the three soils. All the statistical tests were carried out by with the Statgraphics Centurion and XLSTAT software.

3. Results

According to the three-way ANOVA, each individual factor (treatment, soil depth and measurement date) significantly ($p < 0.001$) influences SWR. Similarly, all interactions between couples of factors (soil depth x treatment, time x treatment and soil depth x time, although at $p < 0.01$) are significant to explain the SWR variations. Conversely, the interaction among all the factors (soil depth x treatment x time) is not significant (Table 3).

WDPT of the surface samples was in the range 1.39 (SaL soil treated with FW at 21nd day) to 28.53 (SaL soil treated with OMW at 2nd day) seconds. The average WPDT was 4.38 ± 1.27 seconds for land spreading of FW and 9.15 ± 7.64 seconds for soils treated with OMW; the difference between the treatments was significant ($p < 0.001$) (Table 4).

In the sub-surface layer, WDPT varied between 0.58 (SaL soil treated with OMW at the 21nd day) and 10.38 (same soil type and treatment, but at the 2nd day) seconds. For the deeper soil layer, an average WDPT of 3.84 ± 0.86 seconds was measured for FW treatments. This value was significantly different compared to the WDPT of the soils irrigated with OMW (4.84 ± 2.33 seconds). Based on these WDPT values, the investigated soils can be classified as "wetable" or "slightly water-repellent"(Table 4).

283 Table 3 - Results of the three-way ANOVA analyses applied to soil samples to measure SWR after
 284 OMW and FW land spreading.

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Factors	Degrees of freedom	F-Ratio	P-Value
Soil depth	1	23.32	< 0.001
Time	3	17.11	< 0.001
Treatment	1	32.97	< 0.001
Interactions among factors			
Soil depth x Time	3	4.21	< 0.01
Soil depth x Treatment	1	14.16	< 0.001
Time x Treatment	3	12.74	< 0.001
Soil depth x Time x Treatment	3	3.77	0.13

286 Notes: soil depth = surface layer vs sub-surface layer; time = two vs seven vs fourteen vs twenty-one days after land
 287 spreading; treatment = olive oil mill wastewater vs clear water.

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290 Tables 4a and 4b - Values of WDPT (seconds) and related SWR class over time in soils irrigated with OMW (a) and FW (b) at two depths (mean
291 and std. dev., n = 3).

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(a, treatment with OMW)

Time	Soil layer	Soil type			
		<i>SaL</i>	<i>SiL</i>	<i>SCL</i>	<i>All soil types</i>
		<i>WDPT</i>			<i>SWR class</i>
T_2	SL	28.53 ± 0.49 aA	7.52 ± 0.29 aA	7.82 ± 0.01 aA	slightly repellent
	SSL	10.38 ± 0.10 bA	6.07 ± 0.61 bA	6.11 ± 0.04 aA	
T_7	SL	19.25 ± 0.02 aA	10.82 ± 0.15 aA	11.03 ± 0.74 aA	
	SSL	5.30 ± 0.20 bA	5.22 ± 0.70 bA	5.42 ± 1.12 aA	
T_{14}	SL	4.75 ± 0.02 aA	4.45 ± 0.11 aA	4.94 ± 0.57 aA	wetttable
	SSL	2.70 ± 0.09 aA	3.90 ± 0.50 aA	3.91 ± 0.50 aA	
T_{21}	SL	2.44 ± 0.27 aA	4.13 ± 0.01 aA	4.18 ± 0.05 aA	
	SSL	0.58 ± 0.06 aA	4.09 ± 0.11 aA	4.37 ± 0.16 aA	

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(b, treatment with FW)

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Time	Soil layer	Soil type				
		<i>SaL</i>		<i>SiL</i>	<i>SCL</i>	<i>SiL and SCL soil types</i>
		<i>WDPT</i>	<i>SWR class</i>	<i>WDPT</i>		<i>SWR class</i>
T_2	SL	4.16 ± 0.01 aB	wetable	5.12 ± 0.02 aB	5.15 ± 0.01 aB	slightly repellent
	SSL	3.42 ± 0.01 bA		4.40 ± 0.02 bA	4.41 ± 0.02 aA	wetable
T_7	SL	3.18 ± 0.01 aB		5.15 ± 0.04 aB	5.18 ± 0.01 aB	slightly repellent
	SSL	3.08 ± 0.01 bA		4.42 ± 0.06 bA	4.45 ± 0.02 aA	wetable
T_{14}	SL	2.70 ± 0.21 aA		5.12 ± 0.02 aA	5.15 ± 0.01 aA	slightly repellent
	SSL	2.15 ± 0.01 aA		4.27 ± 0.01 aA	4.32 ± 0.01 aA	wetable
T_{21}	SL	1.39 ± 0.25 aA		5.11 ± 0.01 aA	5.12 ± 0.01 aA	slightly repellent
	SSL	2.36 ± 0.11 aA		4.41 ± 0.01 aA	4.42 ± 0.01 aA	wetable

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Notes: SaL = sandy loam; SiL = silt loam; SCL = silty clay loam; SL = surface soil; SSL = sub-surface layer; OMW = olive oil mill wastewater; FW = clear water; different

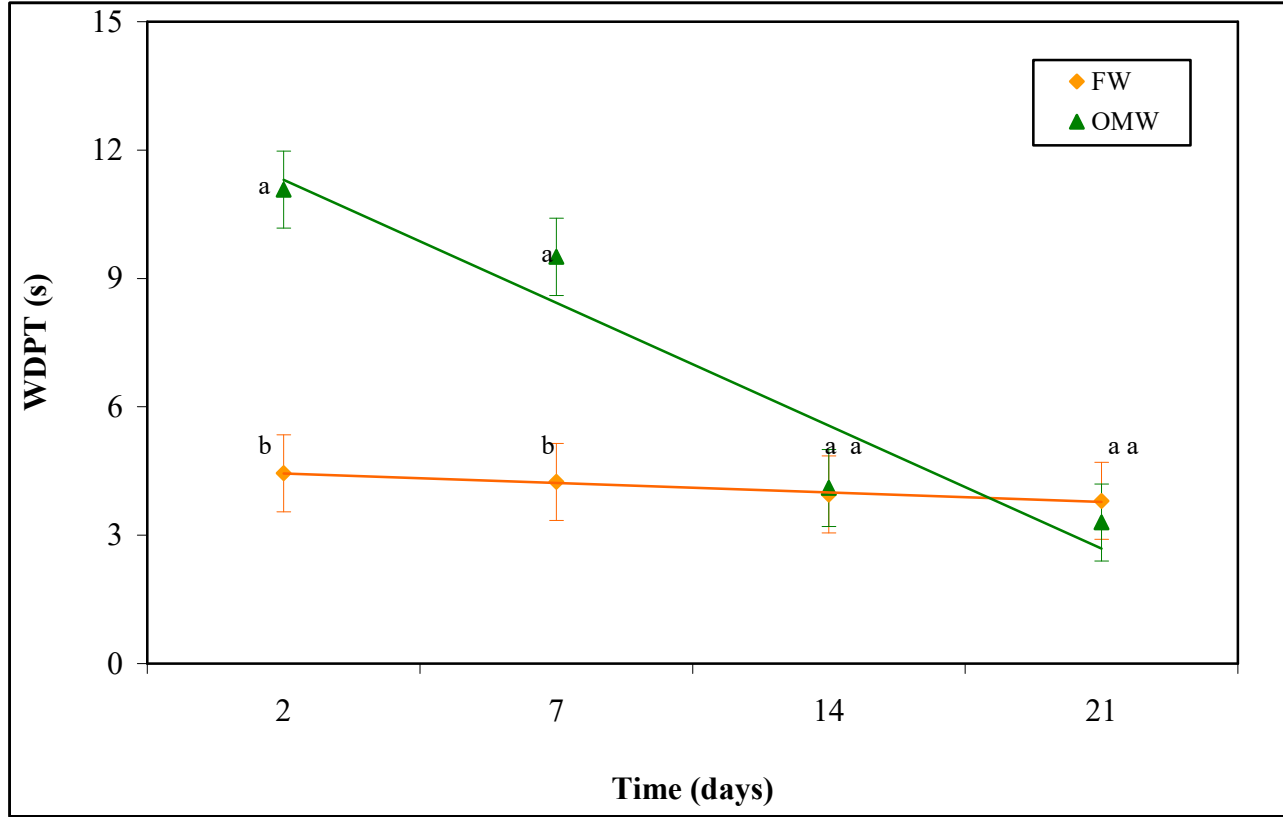
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lowercase and capital letters indicate significant differences after Tukey's test ($p < 0.001$) between SL and SSL as well as OMW and FW, respectively.

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3.1. SWR variations with treatments

The WDPT values of soils irrigated with OMW were significantly higher (about 2-fold, $p < 0.001$) compared to FW treatment until the first week. Due to this SWR increase, the soils became slightly repellent. However, their SWR decreased over time and the soils became not repellent in the following two weeks. The final WDPT of soils treated with OMW was very close to the values of the soils irrigated with FW and the differences (lower than 15%) were not significant (Figure 2).



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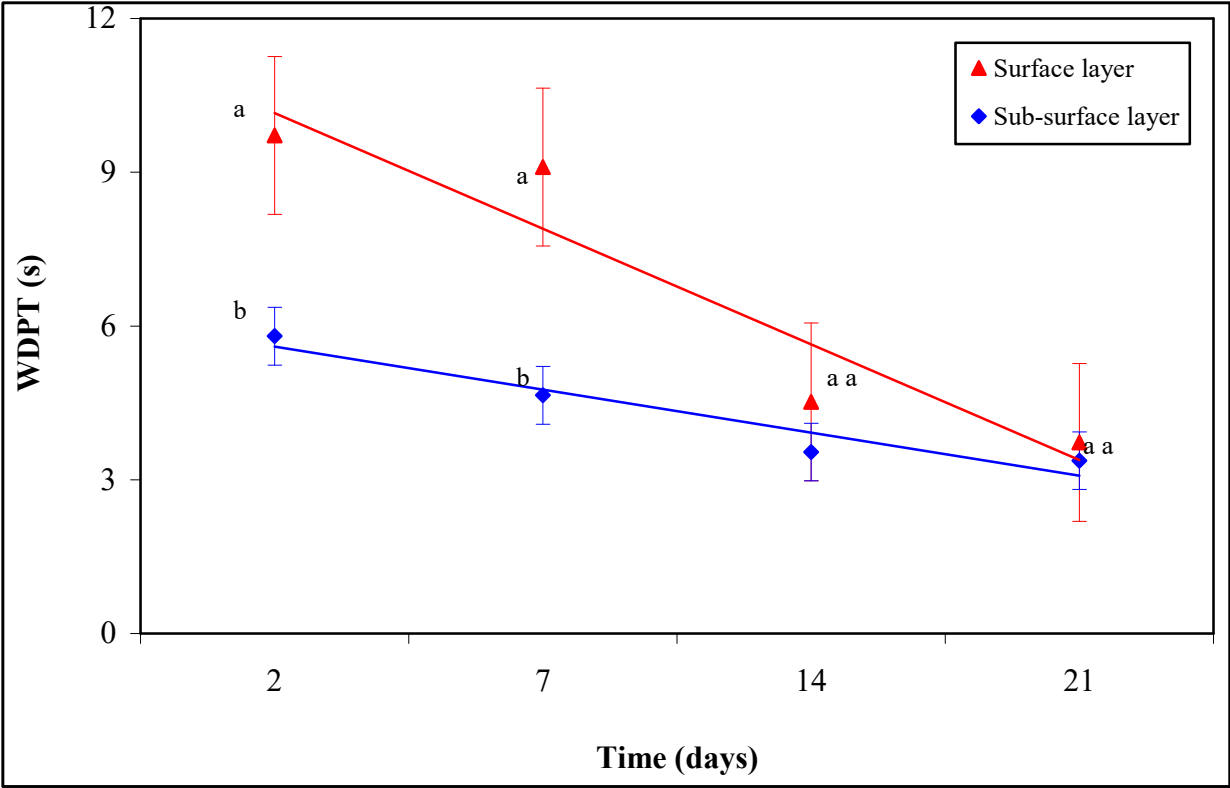
Figure 2 - Interactions between treatment and time factors of three-way ANOVA applied to SWR tests (WDPT = water drop penetration test) (different letters indicate significant differences after Tukey's test ($p < 0.001$)).

3.2. SWR variations with soil depth

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321

The surface layer was always more repellent compared to the sub-surface soil, and this effect was more significant in the first two weeks, when the surface WDPT values were about 50% higher. In the following two weeks, SWR of the two layers became very close and the differences were not

322 significant, although the surface soil was slightly more repellent (differences in WDPT of about
 323 15%) (Figure 3). In more detail, the surface layer was slightly repellent for both treatments until the
 324 second week and became wettable when irrigated with OMW. The sub-surface soil was instead
 325 always wettable, except for the first two weeks after OMW land spreading, when a slight repellency
 326 was noticed (Table 4). More specifically, the control soils, after FW land spreading, was wettable in
 327 the sub-surface layer and slightly water repellent in the surface layer throughout the experiment.
 328 Conversely, both the layers of the soils treated with OMW showed a slight repellency throughout
 329 the first two weeks after irrigation, but became wettable in the following period (Table 4).



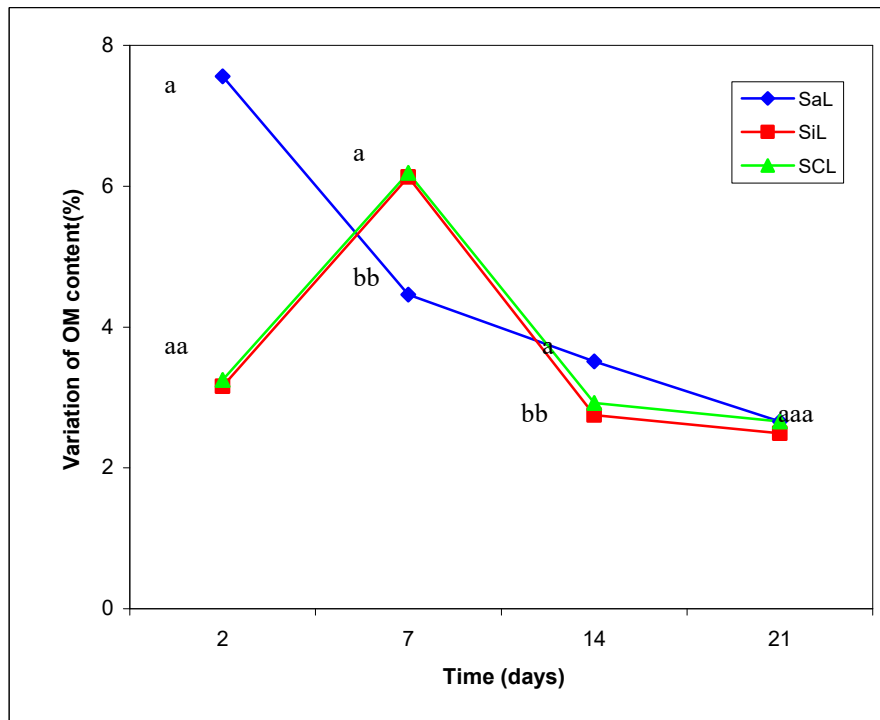
332
 333 Figure 3 - Interactions between soil depth and time factors of three-way ANOVA applied to SWR
 334 tests (WDPT = water drop penetration test) (different letters indicate significant differences after
 335 Tukey's test ($p < 0.001$).

337 3.3. *Effects of OM soil content on SWR*

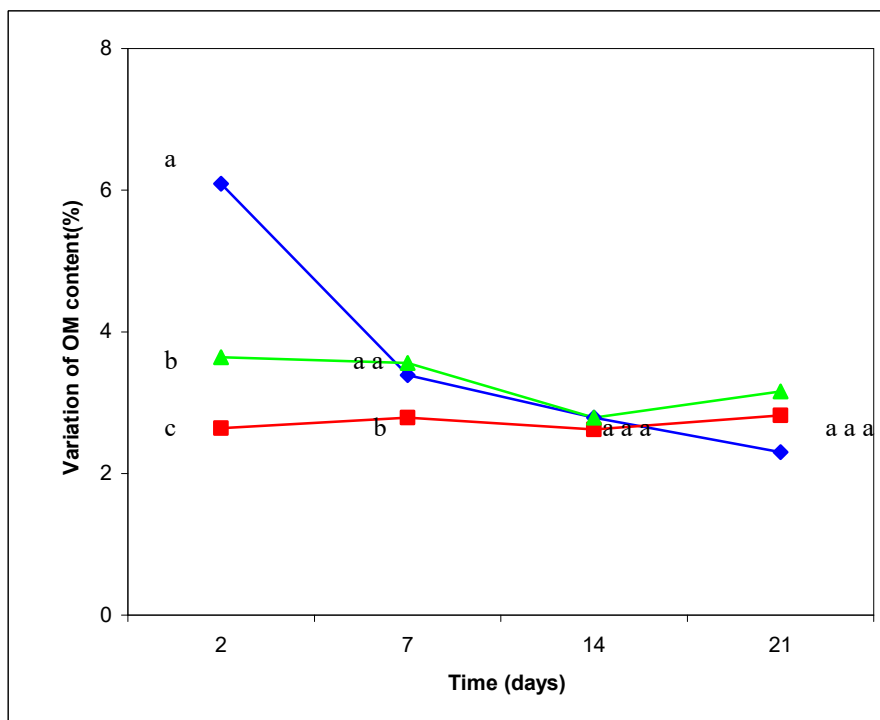
338

339 Immediately after OMW application, the increase in OM content of both surface and sub-surface
340 layers was higher in the SaL soil (respectively, 7.6 and 6.1%). Subsequently, the increase in OM
341 due to OMW application became similar among the three soil types (2.5-2.6% at the soil surface,
342 2.3-3.2% at 10 cm). For the sub-surface layer, OM content of soils showed a low variability over
343 time, although sudden increases (mainly in the surface layer) was observed one week after OMW
344 application (Figure 4). It should be noticed that, after one month from the soil treatment, the
345 original OM content ($2.80 \pm 0.45\%$ for SaL soil, $3.80 \pm 0.44\%$ for SiL and $1.94 \pm 0.03\%$ for SCL)
346 was increased by percentages between 2.5-2.7% (surface layer) and 2.3-3.2% (sub-surface soil)
347 (Figure 4 and Table 2).

348 Linear regressions with high coefficients of determination ($r^2 > 0.86$) were evident by plotting
349 WDPT against OM content of the three soils. The related equations show that SWR increases with
350 OM; moreover, this increase is very sensitive to the changes in OM content of soil, as shown by the
351 high slope of the regression lines. For the sub-surface layer, these correlations were evident and
352 positive for SaL and SiL soils, while the coefficient of determination was much lower for the SCL
353 soil ($r^2 = 0.21$) (Figure 5).



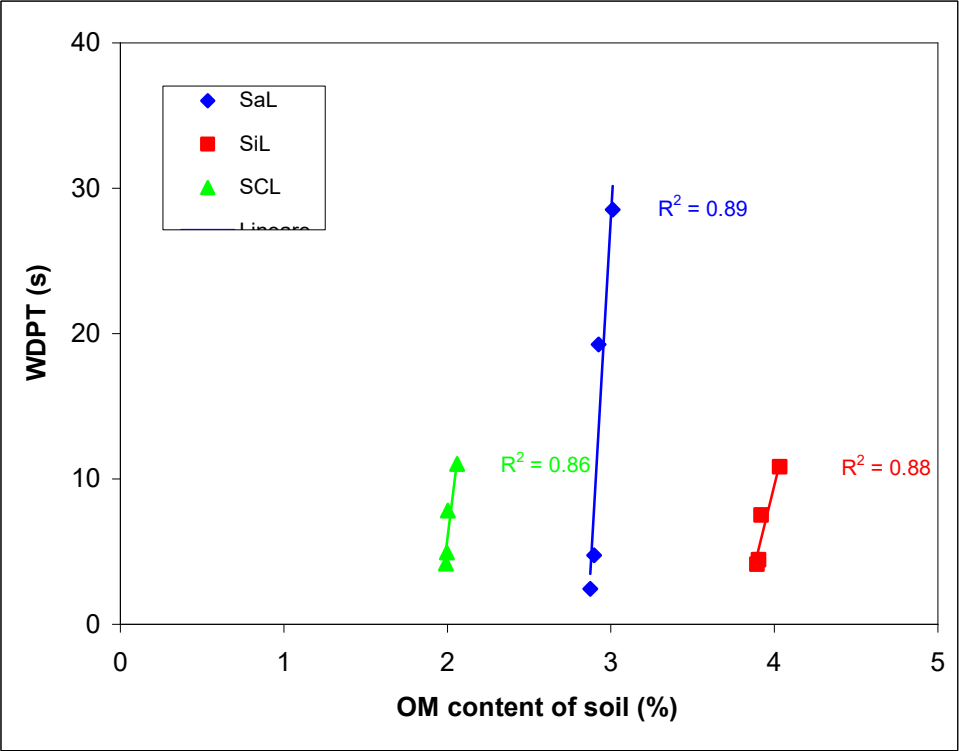
(a)



(b)

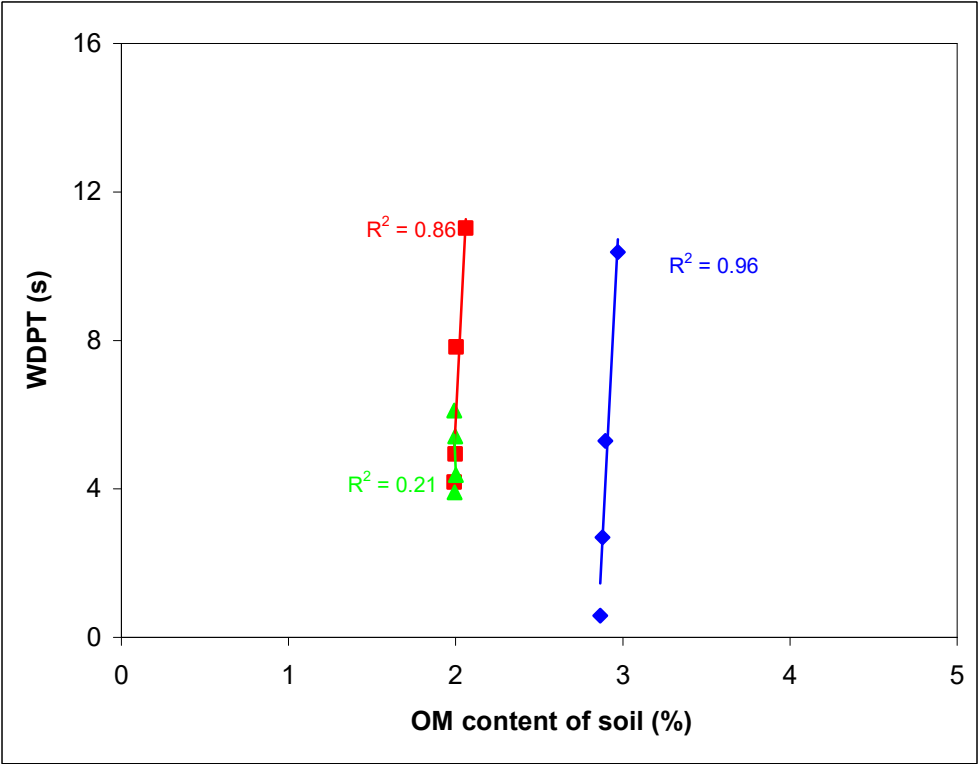
Figure 4 - Variations of organic matter (OM, in percentage over the dry weight) over time compared to the initial value in soils (SaL = sandy loam; SiL = silt loam; SCL = silty clay loam) irrigated with OMW (a, surface layer; b, sub-surface layer) different letters indicate significant differences after Tukey's test ($p < 0.001$).

364
365



366
367
368

(a)



369
370

(b)

371 Figure 5 - Correlations between organic matter (OM) content and Water Drop Penetration Time
372 (WDPT) of the (a) surface layer and (b) sub-surface layer in (SaL) sandy loam, (SiL) silt loam, and
373 (SCL) silty clay loam soils treated with OMW.

374

375 **4. Discussions**

376

377 A large body of literature exists about the evaluation of the effects of wastewater application on
378 SWR. However, much research focussed the long-term effects (from 18 months to even 15 years)
379 and was carried out at field scale, as shown by the main experiences reported in Table 5.

380

381 Table 5 - Main literature experiences on water repellency tests on soils treated with OMW.

382

Reference	Soil texture	Observation period	Land spreading period or frequency	OMW volume applied [m ³ ha ⁻¹]	Time after OMW application	Soil sampling depth [cm]	WDPT(s)/SWR class
Mahmoud et al. (2010)	Silt loam	5 years	October to December	According to the annual production	0-1 months	0-30	25.2
		15 years					36.1
Peikert et al. (2015)	Clay loam	6-18 years	At least one a year	4400	n.a.	0-3	moderate
	Silty loam			5300	n.a.		slight
	Silty loam			1300	n.a.		slight
	Silty clay loam			2000	n.a.		moderate
Steinmetz et al. (2015)	Sandy clay loam	18 months	once a year in winter	70-140*	18 months	0-3	> 5*
			once a year in summer	140*	12 months		> 60-600*
Tamimi et al. (2016)	Clay loam	18 months	single application in Spring	140*	2 days	0-5	0
			single application in Summer – with irrigation				20
			single application in Summer – without				38
						05-ott	13

			irrigation				
			single application in Winter				
			single application in Summer – without irrigation				
			single application in Spring				
Kurtz et al. (2015)	Clayey loam	6 weeks	n.a.	147	3 weeks	0-10	41
	Sandy clay loam						4
Diamantis et al. (2013a)	Sand	37 days	single application	40	37 days	0-5	10% of samples repellent and 60% wettable 72 (50 m ³ ha ⁻¹) to 262 (200 m ³ ha ⁻¹)
Albalasmeh et al. (2019)	Silty loam	2 months	eight weekly applications	50 to 200	n.d.	0-20	
Mohawesh et al. (2019)	Loam	1 month	single application	10 to 120	1 month	0-20	3
	Clay loam					20-40	2-3
This study	Sandy loam	3 weeks	single application	80	2 days	0-10	28.5
	Silt loam						7.5

	Silty clay loam				7 days		7.8
	Sandy loam						19.24
	Silt loam						10.82
	Silty clay loam						11.02

Note: * Estimated value from the reported data.

383
384

385 This study has instead evaluated the degree of soil hydrophobicity determined by OMW few weeks
386 after land spreading. This evaluation is very important to avoid decaying of soil hydraulic properties
387 and thus to control the runoff and erosion risks in the short terms (Barbera et al., 2013; Chatzistathis
388 and Koutsos, 2017; Zema et al., 2019).

389 The effects of OMW land spreading significantly vary with soil profile layer, treatment and time
390 elapsed from application. These variables combine in exerting their influence on soil hydrological
391 response, which also depends on the soil type. More specifically, land spreading of OMW and FW
392 makes the surface layer more repellent compared to the sub-surface soil. FW application never
393 influences soil hydrophobicity in SaL soils, which are not repellent (both in the surface and in the
394 sub-surface layers) over time. This is in accordance with the results of Wallach et al. (2005), who
395 found that land spreading sandy soils with FW does not induce SWR. However, FW induces a
396 slight SWR in the topsoil in SiL and SCL soils throughout the observation period. The increased
397 hydrophobicity of two of our experimental soils could be explained by their higher content of finer
398 soil particles compared to SaL soils. Land spreading induced dispersion of soil particles in the
399 topsoil with consequent formation of a soil crust inducing a slight SWR (Andiloro et al., 2007).

400 Compared to soils irrigated with FW, all soils treated with OMW shows a slight SWR in both layers
401 for about two weeks. This slight SWR affects all soils regardless of their texture. After this period,
402 the soils become wettable. The higher time required for drop penetration in the surface layer in the
403 first two weeks can be attributable to the formation of a lenticular drop of water on the topsoil,
404 which makes the soil surface partially water repellent. Conversely, water infiltrates more rapidly in
405 the wettable soils. The increase in SWR with OMW application has been attributed by Mahmood et
406 al. (2010) to two factors: the generation of hydrophobic components during the decomposition of
407 organic matter, and residues of oil and grease that are wax-like substances forming a coating on soil
408 particles (Bisdom et al., 1993). This coating determines occlusion of the superficial pores due to the
409 chemical composition of the soil solution and the presence of suspended solids (sealing effect)
410 (Barbera et al., 2013; 2014). In more detail, the residual oil in OMW adsorbs onto the soil grains,
411 but, with decreasing particle size (i.e. in clayey soil), the dispersed clay particles coat the
412 hydrophobic compounds responsible for SWR and the soil becomes wettable (Diamantis et al.,
413 2017). Beside the residual oil, the sources of soil hydrophobicity may include plant-derived organic
414 matter (decomposing roots and plant tissues), plant-derived waxes and exudates, fungal activity and
415 microbial products. Moreover, soil contamination by hydrocarbons may induce severe SWR
416 (Sawatski and Li, 1997; Diamantis et al., 2013b). The repulsion of water by the hydrophobic group
417 of organic molecules or are formed during wastewater decomposition in the OMW (Bisdom et al.,
418 1993; Tarchitzky et al., 2007) temporarily reduces the water retention capacity of soils. Therefore,

419 the water availability for plants decreases (Gonzalez-Vila et al., 1995; Wallach et al., 2005; Travis
420 et al., 2008; Mahmoud et al., 2010) and this may be a serious problem for semi-arid climates,
421 characterised by a water shortage in the long and dry season. When the concentration of
422 hydrophobic compounds in the OMW that were rich in oils and fats decrease, soil water retention
423 increases thanks to the hygroscopicity of the organic matter released by OMW and the
424 microporosity improvement in the soil (Barbera et al., 2013). However, this study has demonstrated
425 that much of the OM degradation occurs over time (Piotrowska et al., 2006), decreasing SWR and
426 the previously repellent soil resulted to be wettable after just two weeks. Therefore, this weak
427 hydrophobicity is only temporary and, in short time, the SWR of the soils treated with OMW
428 becomes very similar as the hydrophobicity of soils irrigated with FW (whose water repellency is
429 practically constant over time). Moreover, the SWR of topsoil and sub-surface layer tends to be
430 equal in the short time, as also found by Mohawesh et al. (2019).

431 The high coefficients of determination of the linear regressions between WDPT and OM (with the
432 only exception of the sub-surface layer of the SCL soil) detected in this study confirm the clear
433 influence of OM on SWR and their simultaneous variability in the investigated soils. In general, at
434 the early stage after OMW application, OM noticeably increases in the SaL topsoil. Subsequently
435 the OM content decreases with time, as also observed by Peikert et al. (2015). In the other soil
436 types, OM content is more stable (particularly in the sub-surface layer), which leads to think that the
437 excess of organic compounds applied with OMW is mainly retained in the first centimeters of soil
438 surface without infiltrating, due to lower water infiltrability compared to the SaL soil. The increase
439 after seven days in both SCL and SiL soils is instead quite surprising. This increase may be
440 attributable to a local accumulation of OM, which increases the aggregate stability and
441 macroporosity of soils (Chaney and Swift, 1984; Haynes and Swift, 1990). Preferential water
442 pathway in cracks of these soils with higher clay content, which have been visually detected some
443 days after OMW application. The different soil types may have also affected the microbial activity
444 and therefore the biodegradation rate of hydrophobic compounds in OMW with time. Different
445 researches have demonstrated that soil texture is an important characteristic modulating the
446 microbial communities activity in general (Sessitsch et al., 2001; Bach et al., 2010). For example,
447 clay soils generally support more diverse and greater soil microbial communities compared to sandy
448 soils, as clay better protects the microbial biomass, has got a larger and higher number of soil
449 aggregates and shows a higher water holding capacity (Sessitsch et al., 2001; Six et al., 2006;
450 Voroney, 2007; Wick et al., 2009).

451 The SWR response is consistent to the OM variations in the soil. In other words, SWR decreases
452 with OM. A partial persistence of low SWR with OM contents over the baseline cannot be excluded

(Peikert et al., 2015). However, at the end of the tests, all soils are characterized more or less by similar OM content and SWR class, leading to the conclusion that land spreading of OMW does not noticeably alter OM dynamic and thus soil hydrophobicity for the different soil textures. This main result of this study is in close accordance to the findings of Kurtz et al. (2015). These authors found no repellency ($WDPT < 60$ s) or a slight SWR ($WDPT < 5$ s) in clayey loam or sandy clay loam soils three weeks after OMW land spreading at a 1.5-fold hydraulic load compared to the value of our study (147 against $100 \text{ m}^3/\text{ha}$).

The comparative analysis of the other literature results about SWR determined by land spreading with wastewater shows that:

- (i) the main factor influencing SWR is the OM increase of soil (Wallis and Horne, 1992); more specifically, Nadav et al. (2013a) reported that OM properties have a limited effect on SWR class, while Wallach et al. (2005) concluded that the most influencing factor is OM quantity rather than its quality;
- (ii) SWR occurs mainly in the soil surface layer, independently of the soil texture (Tarchitzky et al., 2007), probably because of the OM content decrease with soil depth (Wallach et al., 2005; Wallis and Horne, 1992);
- (iii) land spreading of wastewater induces noticeable effects on sandy soil (Rusan et al., 2007), due to its lower specific surface area (Nadav et al., 2013a), since the sand particles are more readily coated by OM compared to clay soils (Wallis and Horne, 1992);
- (iv) conversely, SWR effects on clay soil treated with wastewater are less evident (Singh et al., 2012).

Overall, our study has demonstrated that, at least in the experimental conditions, the land application of OMW does not significantly change SWR. In these experiments, the hydraulic and organic loads of the applied water were quite limited compared to the other literature experiences (e.g., Mahmoud et al., 2010; Peikert et al., 2015; Albalasmeh et al., 2019). Limiting the applied volumes does not change SWR and even limits the short-term reduction of soil infiltration rate detected in some studies (e.g., Barbera et al., 2013; Zema et al., 2019). Therefore, the risk of hydrological response worsening immediately after OMW application to soils suggests caution in irrigation of agricultural land, since it may increase runoff and erosion processes. Moreover, according to Steinmetz et al. (2015) and Tamimi et al. (2016), OMW should not be applied to the soil during summer or winter. In summer, the inhibition of degradation of OM constituents and their immobilization in soil at high temperature and low soil moisture after OMW land spreading could be possible (Steinmetz et al., 2015). OMW application in winter, immediately after oil production, although allowing the natural recovery of the soil, could increase the leaching risk due to low

temperature and frequent rains (Tamimi et al., 2016); moreover, in this season, when rainfall amount and intensity are high, the soil is often saturated and the infiltration is lower (Bombino et al., 2010). Therefore, crop irrigation with OMW is preferable during late spring or early autumn, when the irrigation requirement is noticeable, but the rainfall input is lower compared to the wet season; in these periods, biotic degradation of OMW organic compounds is possible, which could reduce the risk of groundwater contamination and enhance biodegradation.

Overall, heavy negative effects on soil hydrophobicity and, more in general, on infiltration characteristics, at least in the experimental conditions, are excluded, if OMW application on soils is practiced following the current Italian limits for OMW spreading on crops. Therefore, OMW land spreading can represent as a viable valorisation approach for these effluents in Mediterranean areas, supporting the olive and oil production chains.

5. Conclusions

In the short-term, the effects of OMW application on soil water repellency significantly vary with depth (surface layer or 10-cm depth) and treatment (land spreading of OMW or FW) of soil as well as with the time elapsed from water application.

Compared to soils treated with FW, OMW determines a slight SWR has affected in both layers of the investigated soils regardless of their texture. However, this weak hydrophobicity is only temporary, since it disappears after two weeks. Evident linear regressions have been found between WDPT and OM content of soils, which show the clear influence of the organic compounds on SWR, decreasing with OM of soil.

From these results, the working hypothesis that the high contents of organic matter and hydrophobic substances of OMW may induce a significant SWR, with consequent worsening of the soil hydraulic properties, should be rejected. Conversely, the study has demonstrated that land spreading OMW does not significantly change SWR, at least at the limited hydraulic and organic loads adopted.

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