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

17

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28

### 29 Abstract

30

31 Land spreading of olive mill wastewater (OMW) allows a cheap and environmentally sound 32 effluent disposal. However, this practice requires suitable application protocols, in order to avoid 33 negative effects on soil hydrological properties, such as the increase of soil water repellency 34 (SWR). The effects of OMW land spreading on SWR, mainly evaluated in the long term, have been 35 rarely measured few days or weeks after land spreading. To this purpose, this study has evaluated 36 the short-term effects of OMW land spreading on SWR of olive groves (with silt, loam or silty clay 37 loam soil texture) using the Water Drop Penetration Test (WDPT) at laboratory scale. SWR 38 significantly (p < 0.001) varied with the soil texture and depth (surface layer or 10-cm depth) as 39 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-40 41 surface layer. Compared to the soils irrigated with FW, a 2-fold WDPT (thus a slightly higher 42 SWR) was found after OMW application in both soil layers, regardless of the texture. However, this 43 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 44 showed the same SWR after four weeks. The high coefficients of determination ( $r^2 > 0.86$ ) in the 45 46 linear regressions between WDPT and OM content of soils proved the clear influence of the organic 47 compounds on SWR, which decreases with OM, as expected. Overall, OMW land spreading has not 48 significantly changed SWR, at least under the limited hydraulic and organic loads adopted in this 49 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

Keywords: Water Drop Penetration Test; soil hydrophobicity; OMW land spreading; soil organic
 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 wet cake, the so-called "olive pomace", and a liquid stream, called "olive mill wastewater" (OMW). 60 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).

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

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

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 degrade soil characteristics (S'habou et al., 2009). The effects of OMW land spreading have been 86 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).

However, literature data about OMW effects on soil properties are not unanimous and, in some cases, contradictory, since the effluents, whose qualitative variability (due to the high variability of pH, and the wide range of concentrations of total solids as well as of organic matter and polyphenols) is large, are applied at several hydraulic rates and over soils of different characteristics (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 (Bisdom 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 numerous, and have been carried out mainly on the long term (e.g. Mahmoud et al. 2010; Peikert et 125 126 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 adverse effects may be present only in this period (Zema et al., 2019). Therefore, there is the need 128 129 of studies evaluating how and by what extent SWR of the Mediterranean soils may change 130 immediately or few weeks after receiving OMW.

To fill this gap, this study evaluates the short-term effects (at 2, 7, 14 and 21 days) of OMW application on SWR of sandy loam, silt loam and silty clay loam soils at different depths (surface and at a depth of 10 cm) at laboratory scale. We hypothesised that the high contents of OM and hydrophobic substances in OMW may noticeably alter SWR, depending on the soil depth and the time elapsed since OMW application. Overall, these short-term effects after OMW land spreading are expected to be negative, leading to the SWR increase, particularly at the soil surface.

137

### 138 **2.** Materials and methods

- 139
- 140 2.1. Study areas
- 141

The investigation was carried out in olive groves (*Olea europea*) of two farms in Calabria, Southern Italy (Figure 1a), of which one is located in Locri and the other in Gioia Tauro. The climate of both farms is typically semi-arid hot-summer Mediterranean climate, Csa class, according to Koppen (1918). The annual rainfall and minimum/maximum temperatures are on average 1300-1400 mm and 11-28 °C, respectively (historical observations of 1923-2017 of Environmental Protection 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
- variability among different textures. One plot, covering an area of 250  $m^2$  (42 m x 6 m), was in
- Locri. The two other plots, of 2 x 360 m<sup>2</sup> (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 theSWR tests (b).
- 165
- 166 According to the USDA/FAO Soil classification (Soil Survey Division Staff, 1993), the soils were
- 167 characterised on 30 to 45 samples per plot (collected at a depth of 10 cm below ground), as:
- 168 (i) sandy loam (on the average 70% w/w of sand, 24% of silt and 6% of clay and 3% of skeleton) in
- 169 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 ofGioia Tauro;
- 172 (iii) silty clay loam (2% of sand, 70% of silt and 28% of clay with 3% of skeleton) in the plot of
- 173 Locri.
- 174 Hereinafter the three soils will be indicated as SaL, SiL and SCL, respectively.
- 175
- 176 2.2. Experimental design

# 178 2.2.1. Soil sampling

179

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 m<sup>2</sup>. The pots were stored in a climatic chamber at 20 °C until the dates of land spreading and SWR measurement.

187 The day after collection, the samples were irrigated with 0.71 litres (about 100 m<sup>3</sup> ha<sup>-1</sup>) of OMW 188 (treated soil) or fresh water (hereinafter, FW). The latter treatment, in which groundwater was used, 189 was considered as control. In this study, the hydraulic load of OMW supply was within the same

190 order of the maximum limit permitted by the Italian law n. 574/96, equal to 80 m<sup>3</sup> ha<sup>-1</sup> per year.

191

192 2.2.2. SWR measurement

193

194 SWR was measured in each pot containing the soil samples at two, seven, fourteen and twenty-one 195 days (henceforth indicated as T<sub>2</sub>, T<sub>7</sub>, T<sub>14</sub> and T<sub>21</sub>, respectively) after land spreading of OMW or FW. 196 According to Letey (1969), the water drop penetration time (WDPT), according to the methods 197 proposed by Van't Woudt in 1959 and commonly accepted in literature (Letey et al., 2000; Buczko 198 and Bens, 2006; Tarchitzky et al., 2007), was used to evaluate SWR. In more detail, two soil 199 samples were gently collected at the measurement dates from the surface layer (SL) and, on the 200 same vertical line, at a depth of 10 cm (sub-surface layer, SSL) of every pot, caring to sampling the 201 soil from a different area of the pot surface at each date. After the measurement, the sampled soils 202 were restored in the pot.

203 The samples were sieved at 2-mm sieve. The material of the samples was filled into circular dishes 204 of 10-cm diameter and the soil surface was manually smoothed. Field-moist samples were used 205 instead of oven-dried soil, in order to measure more realistic values of SWR instead of the 206 "potential" repellency (Buczko and Bens, 2006). Since the samples were collected at the same date 207 from soils with practically the same characteristics, the variability of the water content  $(10.4 \pm 1.1)$ 208 for SaL,  $19.1 \pm 2.4$  for SiL and  $18.3 \pm 1.9\%$  for SCL soil) was very low. Moreover, since all the 209 samples were stored under the same conditions, significant changes in their water content were not 210 expected.

- A total of 10 15 drops of distilled water were applied to the surface of the soil samples through a medical pipette (water volume of one droplet:  $58 \pm 5 \mu$ ) and the WDPT was recorded (Mahmoud et 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)
- slightly water-repellent soil (WDPT = 5 60 s)
- 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

Overall, the experimental design consisted of two treatments (OMW vs FW) x two soil depths (SL 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.

224

### 225 2.2.3. Wastewater and soil characterization

226

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

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

Paramatar	Applied w	Applied water type			
	OMW	FW			
рН (-)	4.14 ± 0.91 a	6.31 ± 0.69 b			
Total suspended solids (mg $l^{1}$ )	5280 ± 190 a	14.0 ± 3.78 b			
Settleable solids (mg $l^{-1}$ )	698 ± 105 b	0 a			
Electrical conductivity (mS $cm^{-1}$ )	1.58 ± 0.70 a	$1.42 \pm 0.51$ a			
$COD (g l^{1})$	12.7 ± 3.68 b	0 a			
Total nitrogen (mg $l^{1}$ )	89.0 ± 4.45 a	$1.10 \pm 0.10$ a			
Polyphenols (g $l^{-1}$ )	$0.65 \pm 0.36$ b	0 a			

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

238

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

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

248

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

250

Paramatar	Soil type					
	SaL	SiL	SCL			
рН (-)	$5.80 \pm 0.09$ a	$5.50 \pm 0.08$ a	$8.20\pm0.16~b$			
OM content (%)	$2.80 \pm 0.45$ ab	$3.80\pm0.44~b$	$1.94 \pm 0.03$ a			
Total carbon (%)	$1.60 \pm 0.10 \text{ ab}$	$2.20\pm0.11~\text{b}$	$1.13 \pm 0.02$ a			
Total nitrogen (%)	$0.90\pm0.03~b$	$2.60 \pm 0.13$ c	$0.19 \pm 0.01$ a			

251 Notes: SaL = sandy loam; SiL = silt loam; SCL = silty clay loam; OM = Organic Matter; different lowercase letters

 $252 \qquad \text{indicate significant differences after t-test at p-level} < 0.05.$ 

The statistical analysis was carried out using the three-way ANOVA. Treatment (OMW or FW), 256 257 soil depth (SL or SSL) and time  $(T_2, T_7, T_{15} \text{ or } T_{21})$  were chosen as factors, while SWR was considered as the response variable. The pairwise comparison by Tukey's test (at p < 0.001) was 258 259 also used to evaluate the statistical significance of the differences in SWR among factors. In order 260 to satisfy the assumptions of the statistical tests (equality of variance and normal distribution), the 261 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 262 263 statistical tests were carried out by with the Statgraphics Centurion and XLSTAT software.

264

### **3. Results**

266

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  $21^{nd}$  day) to 28.53 (SaL soil treated with OMW at  $2^{nd}$  day) seconds. The average WPDT was  $4.38 \pm 1.27$  seconds for land spreading of FW and  $9.15 \pm 7.64$  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  $21^{nd}$  day) and 10.38 (same soil type and treatment, but at the  $2^{nd}$  day) seconds. For the deeper soil layer, an average WDPT of  $3.84 \pm 0.86$  seconds was measured for FW treatments. This value was significantly different compared to the WDPT of the soils irrigated with OMW ( $4.84 \pm 2.33$ seconds). Based on these WDPT values, the investigated soils can be classified as "wettable" 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.
- 285

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.

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 and std. dev., n = 3).

- 292
- 293

294

# (a, treatment with OMW)

Soil type Time Soil layer SaL SiL SCL All soil types WDPT SWR class  $28.53 \pm 0.49 \text{ aA}$  $7.82 \pm 0.01 \text{ aA}$ SL  $7.52 \pm 0.29 \text{ aA}$  $T_2$  $10.38\pm0.10\ bA$  $6.07\pm0.61~bA$  $6.11 \pm 0.04 \text{ aA}$ SSL slightly repellent SL  $19.25 \pm 0.02 \text{ aA}$  $10.82 \pm 0.15 \text{ aA}$  $11.03 \pm 0.74$  aA  $T_7$ SSL  $5.30\pm0.20\ bA$  $5.22\pm0.70\ bA$  $5.42 \pm 1.12 \text{ aA}$  $4.75\pm0.02~aA$  $4.45 \pm 0.11 \text{ aA}$ SL  $4.94 \pm 0.57 \text{ aA}$  $T_{14}$ SSL  $2.70 \pm 0.09 \text{ aA}$  $3.90\pm0.50\;aA$  $3.91\pm0.50~aA$ wettable  $4.18 \pm 0.05 \text{ aA}$ SL  $2.44 \pm 0.27 \text{ aA}$  $4.13 \pm 0.01 \text{ aA}$  $T_{21}$  $4.09 \pm 0.11$  aA SSL  $4.37 \pm 0.16 \text{ aA}$  $0.58\pm0.06\;aA$ 

295

		Soil type							
Time	Soil layer	SaL		SiL	SCL	SiL and SCL soil types			
		WDPT	SWR class	WI	DPT	SWR class			
<i>T</i> .	SL	$4.16\pm0.01~aB$	wettable	$5.12\pm0.02~aB$	$5.15 \pm 0.01 \text{ aB}$	slightly repellent			
12	SSL	$3.42\pm0.01~bA$		$4.40\pm0.02~bA$	$4.41 \pm 0.02 \text{ aA}$	wettable			
<i>T</i> _	SL	$3.18\pm0.01~aB$		$5.15\pm0.04~aB$	$5.18 \pm 0.01 \text{ aB}$	slightly repellent			
17	SSL	$3.08\pm0.01~bA$		$4.42\pm0.06~bA$	$4.45 \pm 0.02 \text{ aA}$	wettable			
TL	SL	$2.70 \pm 0.21 \text{ aA}$	wettable	$5.12 \pm 0.02 \text{ aA}$	$5.15 \pm 0.01 \text{ aA}$	slightly repellent			
1 14	SSL	$2.15 \pm 0.01 \text{ aA}$		$4.27 \pm 0.01 \text{ aA}$	$4.32 \pm 0.01 \text{ aA}$	wettable			
Tal	SL	$1.39 \pm 0.25 \text{ aA}$		5.11 ± 0.01 aA	$5.12 \pm 0.01 \text{ aA}$	slightly repellent			
1 21	SSL	2.36 ± 0.11aA	1	$4.41 \pm 0.01 \text{ aA}$	$4.42 \pm 0.01$ aA	wettable			

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 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.

#### 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).



313

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

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

- 330
- 331



332

Figure 3 - Interactions between soil depth 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).

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, 2.3-3.2% at 10 cm). For the sub-surface layer, OM content of soils showed a low variability over 342 343 time, although sudden increases(mainly in the surface layer) was observed one week after OMW application (Figure 4). It should be noticed that, after one month from the soil treatment, the 344 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) 345 was increased by percentages between 2.5-2.7% (surface layer) and 2.3-3.2% (sub-surface soil) 346 347 (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).



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). 





(b)

- Figure 5 Correlations between organic matter (OM) content and Water Drop Penetration Time
  (WDPT) of the (a) surface layer and (b) sub-surface layer in (SaL) sandy loam, (SiL) silt loam, and
  (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)

and was carried out at field scale, as shown by the main experiences reported in Table 5.

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

Reference	Soil texture	Observation period	Land spreading period or frequency	OMW volume applied [m <sup>3</sup> ha <sup>-1</sup> ]	Time after OMW application	Soil sampling depth [cm]	WDPT(s)/SWR class
Mahmoud et	Silt loam	5 years	October to December	According to the	0-1 months	0-30	25.2
al. (2010)	Sint iouin	15 years		annual production	0 I monuis	0.50	36.1
	Clay loam			4400	n.a.		moderate
Deilvert et al	Silty loam			5300	n.a.		slight
(2015)	Silty loam	6-18 years	At least one a year	1300	n.a.	0-3	slight
	Silty clay loam			2000	n.a.		moderate
Steinmetz et	Sandy clay		once a year in winter	70-140*	18 months		> 5*
al. (2015)	loam	loam	once a year in summer	140*	12 months	0-3	> 60-600*
Tamimietal. (2016)	Clay loam	18 months	single application in Spring	140*	2 days		0
			single application in Summer – with irrigation			0-5	20
			single application in				38
			Summer – without			05-ott	13

			irrigation				
			single application in Winter			0-5	120
			single application in Summer – without irrigation	140*	Months	0-5	46
			single application in Spring				10
Kurtz et al.	Clayey loam						41
(2015)	Sandy clay loam	6 weeks	n.a.	147	3 weeks	0-10	4
Diamantis et al. (2013a)	Sand	37 days	single application	40	37 days	0-5	10% of samples repellent and 60% wettable
Albalasmeh et al. (2019)	Silty loam	2 months	eight weekly applications	50 to 200	n.d.	0-20	72 (50 m <sup>3</sup> ha <sup>-1</sup> ) to 262 (200 m <sup>3</sup> ha <sup>-1</sup> )
Mohawesh	Loam	1 month	single application	10 to 120	1 month	0-20	3
et al. (2019)	Clay loam					20-40	2-3
This study	Sandy loam	3 weeks	single application	80	2 days	0-10	28.5
	Silt loam	1			1		75

Silty	clay			78
loar	m			7.0
Sandy	loam			19.24
Silt lo	bam		7 days	10.82
Silty	clay		, augo	11.02
loa	m			

383 Note: \* Estimated value from the reported data.

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

- 453 (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
- 455 noticeably alter OM dynamic and thus soil hydrophobicity for the different soil textures. This main
- 456 result of this study is in close accordance to the findings of Kurtz et al. (2015). These authors found
- 457 no repellency (WDPT < 60 s) or a slight SWR (WDPT < 5 s) in clayey loam or sandy clay loam
- 458 soils three weeks after OMW land spreading at a 1.5-fold hydraulic load compared to the value of
- 459 our study (147 against  $100 \text{ m}^3/\text{ha}$ ).
- 460 The comparative analysis of the other literature results about SWR determined by land spreading461 with wastewater shows that:
- 462 (i) the main factor influencing SWR is the OM increase of soil (Wallis and Horne, 1992); more
  463 specifically, Nadav et al. (2013a) reported that OM properties have a limited effect on SWR
  464 class, while Wallach et al. (2005) concluded that the most influencing factor is OM quantity
  465 rather that its quality;
- 466 (ii) SWR occurs mainly in the soil surface layer, independently of the soil texture (Tarchitzky et
  467 al., 2007), probably because of the OM content decrease with soil depth (Wallach et al., 2005;
  468 Wallis and Horne, 1992);
- 469 (iii) land spreading of wastewater induces noticeable effects on sandy soil (Rusan et al., 2007), due
  470 to its lower specific surface area (Nadav et al., 2013a), since the sand particles are more
  471 readily coated by OM compared to clay soils (Wallis and Horne, 1992);
- 472 (iv) conversely, SWR effects on clay soil treated with wastewater are less evident (Singh et al.,473 2012).
- 474 Overall, our study has demonstrated that, at least in the experimental conditions, the land 475 application of OMW does not significantly change SWR. In these experiments, the hydraulic and 476 organic loads of the applied water were quite limited compared to the other literature experiences 477 (e.g., Mahmoud et al., 2010; Peikert et al., 2015; Albalasmeh et al., 2019). Limiting the applied 478 volumes does not change SWR and even limits the short-term reduction of soil infiltration rate 479 detected in some studies (e.g., Barbera et al., 2013; Zema et al., 2019). Therefore, the risk of 480 hydrological response worsening immediately after OMW application to soils suggests caution in 481 irrigation of agricultural land, since it may increase runoff and erosion processes. Moreover, 482 according to Steinmetz et al. (2015) and Tamimi et al. (2016), OMW should not applied to the soil 483 during summer or winter. In summer, the inhibition of degradation of OM constituents and their 484 immobilization in soil at high temperature and low soil moisture after OMW land spreading could 485 be possible (Steinmetz et al., 2015). OMW application in winter, immediately after oil production, 486 although allowing the natural recovery of the soil, could increase the leaching risk due to low

487 temperature and frequent rains (Tamimi et al., 2016); moreover, in this season, when rainfall 488 amount and intensity are high, the soil is often saturated and the infiltration is lower (Bombino et 489 al., 2010). Therefore, crop irrigation with OMW is preferable during late spring or early autumn, 490 when the irrigation requirement is noticeable, but the rainfall input is lower compared to the wet 491 season; in these periods, biotic degradation of OMW organic compounds is possible, which could 492 reduce the risk of groundwater contamination and enhance biodegradation.

493 Overall, heavy negative effects on soil hydrophobicity and, more in general, on infiltration 494 characteristics, at least in the experimental conditions, are excluded, if OMW application on soils is 495 practiced following the current Italian limits for OMW spreading on crops. Therefore, OMW land 496 spreading can represent as a viable valorisation approach for these effluents in Mediterranean areas, 497 supporting the olive and oil production chains.

498

## 499 **5.** Conclusions

500

501 In the short-term, the effects of OMW application on soil water repellency significantly vary with 502 depth (surface layer or 10-cm depth) and treatment (land spreading of OMW or FW) of soil as well 503 as with the time elapsed from water application.

504 Compared to soils treated with FW, OMW determines a slight SWR has affected in both layers of 505 the investigated soils regardless of their texture. However, this weak hydrophobicity is only 506 temporary, since it disappears after two weeks. Evident linear regressions have been found between 507 WDPT and OM content of soils, which show the clear influence of the organic compounds on 508 SWR, decreasing with OM of soil.

509 From these results, the working hypothesis that the high contents of organic matter and hydrophobic 510 substances of OMW may induce a significant SWR, with consequent worsening of the soil 511 hydraulic properties, should be rejected. Conversely, the study has demonstrated that land spreading 512 OMW does not significantly change SWR, at least at the limited hydraulic and organic loads 513 adopted.

514

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516

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