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Highlights

► Short- and long-term effects of OMW disposal were assessed on soil properties. ► OMW effects on soil chemical and biochemical parameters were not long-lasting. ► Soil EC, exchangeable K, soluble phenols and ammonium were short-lasting affected. ► OMW disposal increased qCO_2 and reduced microbial biomass and Cmic/Corg. ► OMW disposal reduced AM fungal root colonisation and increased arbuscules.

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Short- and long-term effects of olive mill wastewater land spreading on soil chemical and biological properties

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ABSTRACT

Olive mill wastewater (OMW) is the main residual product of olive processing and its disposal can represent a relevant environmental issue in Mediterranean countries, where olive oil production is large and concentrated in a short-lasting period. OMW is characterised by high pollutant load, salinity and phytotoxic levels of polyphenols, but also by a high amount of organic compounds and plant mineral nutrients. Therefore, OMW field spreading may represent a low cost contribution to crop fertilisation and soil amendment. Here, we assessed the short- and long-term effects of long-lasting repeated OMW applications on soil chemical and biochemical properties and arbuscular mycorrhizal fungi (AMF). In addition the influence of two OMW management strategies, characterised by different seasonality of spreading (autumn and spring times) was evaluated. Soil was amended by 0 and 80 m^3 ha^{-1} of OMW. Principal coordinate analyses (PCO) together with PERMANOVAs showed that long-lasting repeated OMW spreading: (i) affected the main soil chemical and biochemical parameters in the short-term, whereas did not determine long-term residual effects irrespective of the application times; (ii) decreased AM fungal root colonisation both after autumn and spring OMW applications; (iii) improved arbuscule occurrence in the short- and long-term for both disposal times. Therefore, at least regarding the monitored parameters, we can argue that OMW may be utilised as organic amendment in agriculture under controlled conditions given the short-term negative effects on soil quality, which can be considered negligible after a suitable waiting period.

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1. Introduction

Olive-tree plantation is a major agricultural land use in Mediterranean areas, covering in 2007 over 5 million hectares only in the European Union (EU) Member States. The EU dominates the international olive oil market and the main production countries are Spain with 2.47 million ha, followed by Italy (1.16 million ha), Greece (0.81 million ha) and Portugal (0.38 million ha) (López-Piñeiro et al., 2011; Lozano-García et al., 2011). Such Mediterranean countries contribute to more than 70% of the worldwide production of olive oil, estimated at about 3×10^6 tonnes per year (IOOC, 2010). As regards olive processing, olive mill wastewater (OMW) and olive pomace are the main residual products (Kapellakis et al., 2008). The amount of the OMW by-product is estimated between 0.5 and 1.5 m^3 per one tonne of olive and depends on many parameters, such as the olive variety, olive seed maturity, cultivation techniques and pedo-climatic conditions. However, the volume of OMW produced is strongly influenced by the milling method (Sierra et al., 2001; Kapellakis et al., 2008). In Mediterranean areas such wastewaters are produced over a short-lasting period during the winter time, from November to February (Moraetis et al., 2011), and their amount varies between 7 and 30 millions m^3 per year (Cabrera et al., 1996; Kavvadias et al., 2010; López-Piñeiro et al., 2011).

OMW represents 2/3 of mill residues and is composed of 83–92% water naturally present in olives (constitutional water) and water coming from the cleaning (olive and equipment) and extracting operations (technological water) (Alianello, 2001; Bonari and Silvestri, 2003; Kapellakis et al., 2008). Independently of the extraction technology utilised, such residual product represents a serious environmental problem, because it is characterised by large volumes, low pH, salinity, high organic load and amount of toxical-phytotoxical compounds, such as polyphenols (Gamba et al., 2005; Sierra et al., 2007; Federici et al., 2009; Piotrowska et al., 2011).

In Mediterranean countries, where the coastal areas are mainly characterised by arid- and semiarid climatic conditions, scarcity of

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soil organic matter and water deficiency (García et al., 1994; Alianello, 2001), the most suitable OMW use is the so-called *land treatment* and *utilisation* (Cabrera et al., 1996). Actually, although the chemical composition of OMW might cause soil and/or water contamination, its use in agriculture as amendment/fertiliser is promoted because of the high content in mineral nutrients, such as N, P, K, Mg and Fe and the consistent rate of organic matter (OM) (Bonari and Ceccarini, 1991; Alianello, 2001; Mekki et al., 2006a; Lozano-García et al., 2011). In *land treatment/utilisation*, OMWs are spread on soil with or without crops being grown (Levi-Minzi et al., 1992; Cabrera et al., 1996; Sierra et al., 2001; Mekki et al., 2006a; Kapellakis et al., 2008; Mechri et al., 2008).

During the last 30 years, several studies were performed, at laboratory and plot scales, aiming to assess the impact of the direct application of OMW on the fertility of bare soils (Paredes et al., 1987; Levi-Minzi et al., 1992; Cabrera et al., 1996) and on the chemical properties and plant performances of cultivated lands (Mekki et al., 2006a; Mechri et al., 2007, 2011; Arienzo et al., 2009; Chartzoulakis et al., 2010; Lozano-García et al., 2011; Moraetis et al., 2011). The underestimation of key factors, such as soil texture and properties, moisture, water table, type of crop and the uncontrolled spreading were shown to produce negative impacts on soil–plant relationship. However, moderate and controlled OMW land application may result in no negative long-term effects on soil chemical parameters and in improving soil fertility and productivity.

So far, little interest has been given to the impact of OMW land spreading on soil microbial parameters and all the studies performed focused just on the effect of single OMW applications. As regards laboratory scale, Tardioli et al. (1997), observed increments of soil microorganisms and changes in the fungal community at different OMW concentrations, while Bodini et al. (2011), observed a large short-term effect on the bacterial and fungal rhizosphere population one year after OMW spreading, but no changes on microbial biomass carbon (MBC) and total bacteria. As regards field scale, Di Serio et al. (2008) and Mekki et al. (2006b), one and two months after OMW application, observed increases in soil respiration (SR) linked to a "priming effect" induced by the more labile part of the organic matter supplied, whereas Gamba et al. (2005) and Saadi et al. (2007) over three and six months, assessed beneficial effects on MBC and no significant increases in SR and metabolic quotient (qCO_2). As regards soil microbes, few works focused on arbuscular mycorrhizal fungi (AMF) (Mechri et al., 2008; Ipsilantis et al., 2009). AMF (Phylum: Glomeromycota) form mutualistic symbioses with the roots of most land plants and are fundamental for soil fertility, increasing plant growth, nutrient uptake and improving soil structure and protection against biotic and abiotic stressors (Smith and Read, 2008).

In this study we evaluated two strategies of OMW management characterised by a different seasonality of spreading on the basis of the short- and long-term effect of long-lasting repeated OMW field applications. We monitored some reliable and sensitive soil chemical and biochemical parameters, and with regard to the biological ones, AMF.

2. Materials and methods

2.1. Study areas

The study was carried out in two olive mills in Tuscany, Central Italy. The first olive mill was located in Caprona (site A; 43°42′N lat, 10°30′E long; with 12 m above sea level), Pisa (northern Tuscany), whereas the second one in Braccagni (site B; 42°52′N lat, 11°40′E long; with 19 m above see level), Grosseto (southern Tuscany). The soil of site A and site B were classified as silty clay loam (sand 14%, silt 53%, clay 33%) and sandy loam (sand 58%, silt 26%, clay 16%), respectively, according to the USDA system (USDA-NRCS, 1996) and showed values of bulk density of 1.29 and 1.47 g cm^{-3}, respectively. Details of the physical characteristics of the OMW amended soils belonging to the two sites are shown in Table 1, together with the characteristics of the unamended ones (controls). Climatic conditions were typically Mediterranean: rainfall mainly concentrated from autumn to spring (mean 950 mm $year^{-1}$) and mean monthly air temperature ranging from 4 to 10 °C in February to 20–25 °C in July (mean of 15 °C $year^{-1}$).

Both olive mills have a 3-phase production system based on the continuous centrifuge extraction process with a mean working capacity in olive of three tonnes per hour. In site B they were concentrated from the second week of October to the end of December. Site A stores the OMW until the end of the milling process (March) and the distribution is restricted to April and May. On the contrary, in site B the OMW distribution is continuous, starting in November until mid-January and therefore the storage period is shorter than in site A. Both sites have been used for the disposal of OMW for five years and OMWs were incorporated into the soil by harrowing (20 cm depth). The soils were uncultivated and left to develop under the natural succession vegetation. In site A the main plant species were white clover (*Trifolium repens* L.), bear garlic (*Alium ursinum* L.), bitter lettuce (*Lactuca virosa*), perennial ryegrass (*Lolium perenne* L.) and creeping cinquefoil (*Potentilla reptans* L.), while in site B were black mustard (*Brassica nigra* (L.) W.D.J. Koch), common beet (*Beta maritima* L.) and perennial ryegrass.

2.2. Experimental design and OMW application

The OMWs used in the present study were collected from site A and site B and their main properties are shown in Table 2. In both sites, the experimental design was completely randomised with two OMW treatments, two sampling times and three replicates ($n = 3$; field plots of 500 m^2). The OMW treatments were: (1) OMW amended soil: 80 m^3 ha^{-1}, corresponding to the highest amount allowed by Italian law (n. 574, G.U. n. 265/1996); (2) unamended soil (control): soil where OMW was never spread. In order to evaluate the length and the range of OMW spreading effects we choose two sampling times: short-term and highest expected effects (time 1): five days after spreading; long-term and residual/lowest expected effects (time 2): six months after spreading.

2.3. Sampling

At site A, in May and November 2010 (five days and six months after OMW spreading), one combined soil sample, obtained from three random soil cores pooled together, was collected from each field replicate plot (0–20 cm depth). At site B, in November 2010 and in May 2011 (five days and six months after OMW spreading), the soil was similarly sampled. Soil samples utilised for chemical analyses were oven dried at 30 °C and sieved at 2 mm, whereas samples used for biochemical analyses were sieved at field moisture. Soil and roots of perennial ryegrass, identified as the co-occurring plant species, were sampled by extracting turfs ($n = 3$)

Table 1
Initial soil physical properties of control and olive mill wastewater amended soils belonging to two olive mills (site A and site B) at a depth of 0–20 cm.

Properties	Site A[a]		Site B	
	Control	OMW	Control	OMW
Sand (%)	14.34 ± 1.54[b]	14.02 ± 1.45	60.10 ± 0.82	55.68 ± 2.20[c]
Silt (%)	54.21 ± 1.02	51.94 ± 0.30	23.93 ± 1.04	27.18 ± 1.86
Clay (%)	31.45 ± 0.75	34.03 ± 1.17	15.98 ± 0.68	17.14 ± 0.46

[a] Site A: Caprona, Pisa, Italy; Site B: Braccagni, Grosseto, Italy.
[b] Means ± SE of four replicates.

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Table 2
Main properties of the olive mill wastewaters obtained from two olive mills (site A and site B) and utilised as amendment in each corresponding soil.

Properties[a]	Site A[b]	Site B
pH (H_2O, 1:2.5)	4.68 ± 0.03[c]	5.53 ± 0.78
EC (mS cm^{-1})	7.33 ± 0.05	6.58 ± 0.05
Ca_{tot} (mg kg^{-1})	189.20 ± 11.65	190.50 ± 7.01
Ca_{sol} (mg kg^{-1})	112.00 ± 2.16	158.25 ± 1.93
Mg_{tot} (mg kg^{-1})	98.25 ± 1.25	110.00 ± 0.41
Mg_{sol} (mg kg^{-1})	83.00 ± 1.41	96.25 ± 1.11
K_{tot} (g kg^{-1})	3.40 ± 0.05	2.95 ± 0.26
K_{sol} (g kg^{-1})	3.32 ± 0.06	2.67 ± 0.23
N_{tot}(g kg^{-1})	1.40 ± 0.19	0.50 ± 0.12
P_{tot} (mg kg^{-1})	267,025 ± 2.81	214.25 ± 1.93
BOD_5 (mg O_2 L^{-1})	56,575.00 ± 6089.52	32,175.00 ± 5860.12
COD (mg O_2 L^{-1})	127,920.00 ± 16,424.11	80,240.03 ± 17,452.66
Phenols (mg coumaric acid kg^{-1})	3594.50 ± 192.19	3432.00 ± 60.61

[a] EC: electrical conductivity; Ca_{tot}: total calcium; Ca_{sol}: soluble calcium; Mg_{tot} total magnaesium; Mg_{sol}: soluble mangesium; K_{tot}: total potassium; K_{sol}: soluble potassium; N_{tot}: total nitrogen; P_{tot}: total phosphorus; BOD_5: biological oxygen demand; COD: chemical oxygen demand.
[b] Site A: Caprona, Pisa, Italy; Site B: Braccagni, Grosseto, Italy.
[c] Means ± SE of four replicates.

of approximately 7–10 cm across and 20 cm deep from all the plots. In the laboratory each turf was carefully washed in water in order to avoid root breaking. Only the fine roots attached to the main roots of the perennial ryegrass were collected and utilised to assess AM fungal root colonisation.

2.4. Soil chemical, biochemical and AM fungal analyses

Soil samples were analysed for: pH; electrical conductivity, EC; exchangeable potassium, K_{exch}; total nitrogen, N_{tot}; nitrate, NO_3^-; ammonium, NH_4^+; organic carbon, SOC; total phosphorus, P_{tot}; available phosphorus, P_{avail}; soluble phenols; microbial biomass carbon, MBC; soil respiration, SR and AM fungal colonisation. All these analyses were carried out in three replicates. Soil pH and EC were measured in deionised water (1:2.5 and 1:2 w/v, respectively) (McLean, 1982). K_{exch} was determined using the atomic absorption (Thomas, 1982). N_{tot} was evaluated by macro Kjeldahl digestion procedure (Bremner and Mulvaney, 1982), while NO_3^- and NH_4^+ by the Keeney and Nelson method (1982). SOC was measured using the modified Walkley-Black wet combustion method (Nelson and Sommers, 1982). P_{tot} and P_{avail} were determined by colorimetry using a perchloric acid digestion and a solution of sodium bicarbonate, respectively (Olsen and Sommers, 1982). Gravimetric method was used to determine soil soluble phenols after extraction by Folin-Ciocalteau reagent (Carter and Gregorich, 2008; Chantigny et al., 2008). MBC and SR were assessed on soil samples adjusted to 55% of the field water capacity. In detail, MBC was determined by the Vance chloroform fumigation-extraction method, while SR was estimated using the Isermeyer method (Alef and Nannipieri, 1995). Ten-days after incubation in closed jars maintained at 25 °C, MBC and SR parameters were assessed by titration on subsamples of 60 g and 45 g, respectively. The CO_2 evolved during the 10th day of incubation is commonly used as the basal respiration value because, after that period, the soil reached a relatively constant hourly CO_2 production rate (Moscatelli et al., 2005).

The microbial quotient (Cmic/Corg) was calculated as the ratio between MBC and SOC, while the metabolic quotient (qCO_2) between SR and MBC. Such indices were used to describe the microbial biomass contribution to SOC and SR, respectively (Anderson and Domsch, 1989).

AM fungal root colonisation was determined under a stereomicroscope (Olympus SZX 9, Olympus Optics, Tokyo, Japan), after clearing and staining with lactic acid instead of phenol (Phillips and Hayman, 1970), following the gridline intersect method (Giovannetti and Mosse, 1980). Then, the roots were mounted on microscope slides and examined, and arbuscules and vesicles were determined at magnifications of ×125–500 and verified at a magnification of ×1250 using an optical microscope (Leitz Laborlux S, Wetzlar, Germany) following the magnified intersection method (McGonigle et al., 1990).

2.5. Statistical analyses

The data were analysed by a mixed model two-way nested analysis of variance (ANOVA), using OMW treatment and sampling time as factors, respectively. In detail, sampling time (time) was used as the fixed factor and OMW treatment as the random factor nested within time. In addition, the data were analysed by one-way ANOVA using OMW treatment and time as the factors, and the means were compared by the *t*-test. In both analyses, the data were ln- or arcsine transformed when it was necessary to fulfil the assumptions of ANOVA, which was carried out according to the completely randomised design. Means and standard errors given in the tables are for untransformed data. All the analyses were performed using SPSS 17.0 software (SPSS Inc., Chicago, IL, USA).

Moreover, the dataset of each site was square root transformed and Bray–Curtis' coefficients of similarity were calculated between samples in order to compute the principal coordinates analysis (PCO; Torgerson, 1958), allowing the observation of the most relevant patterns and structures. PCO was performed on Primer 6 & PERMANOVA plus (Clarke and Gorley, 2006; Anderson et al., 2008). This flexible ordination method was chosen because it can be based on any resemblance matrix, projecting the points onto axes that minimise residual variation in the space of the resemblance measure applied. In addition, to test the simultaneous response of all soil parameters, recorded in each site, against the two factors (time: fixed factor; OMW treatment: nested factor within time) in an ANOVA experimental design, the permutational analysis of variance (PERMANOVA) was used (Anderson, 2001). Discrimination was based upon Bray–Curtis similarity and *P*-values were calculated using the Monte Carlo test together with the permutation of residuals under a split-plots model (Anderson and Ter Braak, 2003). Records from the same time were treated as split-plots of the same whole-plot and only the six whole plots were permuted.

3. Results

3.1. Chemical parameters

In the two sites long-lasting repeated OMW land spreading determined differential short- and long-term impacts on soil chemical parameters in comparison with the control (Tables 3, 4). In site A the mixed model two-way nested ANOVA highlighted a significant effect of the OMW treatment on soil pH (Table 3). In detail, at the first sampling time, a statistically significant pH decrease was observed in the amended plots compared with the control, whereas six months after the OMW treatment the amended soils showed higher values of pH than the untreated ones. The *t*-test pointed out a significantly increase of pH in the OMW treated soil from May (time 1) to November (time 2), whereas it decreased in the controls (Table 4). In site B, soil pH changed over time and a significant increase was detected from November (time 1) to May (time 2) in the OMW treated soil and control (Tables 3, 4).

EC was affected by the OMW treatment at both sites, showing at time 1 and at time 2 statistically significant increases and decreases in comparison with control, respectively (Tables 3, 4). In addition, EC decreases were observed in the OMW treated soils over the two

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Table 3
Mixed model two-way nested ANOVAs to detect significant differences on soil chemical, biochemical and arbuscular mycorrhizal (AM) fungal parameters between olive mill wastewater (OMW) treatments (amended soil *vs* control) in two different olive mills (site A and site B) and time, five days and six months after OMW addition.

Soil parameters[a]	Site A[b]		Site B	
	OMW[c]	Time	OMW	Time
Chemical				
pH	**<0.001**[d]	0.496	0.992	**<0.001**
EC	**<0.001**	0.420	**<0.001**	0.412
K_{exch}	**<0.001**	0.412	**<0.001**	0.414
N_{tot}	**<0.001**	0.372	0.227	0.400
NO_3^-	0.856	0.011	**0.047**	0.308
NH_4^+	**<0.001**	0.115	**0.007**	0.785
SOC	**0.005**	0.333	0.140	0.428
P_{tot}	**<0.001**	0.489	**0.001**	0.847
P_{avail}	**<0.001**	0.413	**0.001**	0.665
Soluble phenols	**<0.001**	0.404	**0.003**	0.454
Biochemical				
MBC	**<0.001**	0.901	**<0.001**	0.676
SR	**0.014**	0.516	**0.024**	0.855
Cmic/Corg	**0.005**	0.333	**<0.001**	0.766
qCO_2	**<0.001**	0.476	**0.023**	0.419
AM fungi				
Root colonisation	**0.001**	0.590	0.259	**0.037**
Arbuscules	**0.041**	0.566	**<0.001**	0.741
Vesicles	**0.011**	0.243	0.298	0.765

[a] EC: electrical conductivity; K_{exch}: exchangeable potassium; N_{tot}: total nitrogen; NO_3^-: nitrate; NH_4^+: ammonium; SOC: soil organic carbon; P_{tot}: total phosphorus; P_{avail}: available phosphorus; MBC: microbial biomass carbon; SR: soil respiration; Cmic/Corg: microbial carbon/soil organic carbon ratio; qCO_2: metabolic quotient, SR/MBC.
[b] Site A: Caprona, Pisa, Italy; Site B: Braccagni, Grosseto, Italy.
[c] Mixed model two-way nested design ANOVA: time, fixed factor; OMW treatment: random factor nested within time.
[d] *P*-values of the two-way ANOVA: bold values are significant ($P < 0.05$). Replicate field plots were at least three per treatment.

sampling times (from May to November in site A and from November to May in site B) (Table 4).

K_{exch}, was significantly affected by the OMW treatment at both spreading sites and, just after OMW disposal, their values were found to be four- to 10-fold higher than the observed ones in the control (Tables 3, 4). By contrast, at second sampling, low increases were recorded (a mean of 36%). In addition, at both OMW treated sites, six months after spreading soil K_{exch} values were six-fold lower than the values observed at time 1 (Table 4).

As regard N_{tot}, the OMW treatment determined significant increases only in site A (Table 3). Moreover, at both sites over the two sampling times statistically significant N_{tot} decreases were consistently detected in the OMW treated soils (Table 4).

Soil nitrate concentration was significantly affected by time at site A and by the OMW treatment at site B (Table 3). Interestingly, at the spreading, in site B the mineralised-N was higher in the treated soil than in the control, while at both OMW treated soils significant decreases were detected from spreading to time 2 (Table 4).

As regard soil ammonium, the two-way nested ANOVA highlighted a significant effect of the OMW disposal at both study areas (Table 3). In detail, while at spreading an increase in soil ammonium was recorded at both sites in comparison with the unamended soil, no changes were observed six months later. Moreover, while in site A significant decreases of NH_4^+ were detected from spreading to time 2 both in the amended and unamended soils, in site B NH_4^+ increases were observed only in the control (Table 4).

Here, the OMW treatment significantly affected SOC at site A, producing a strong impact just after disposal (Tables 3, 4). Actually, values four-fold higher than in the control were revealed in the OMW amended plots. In addition, the *t*-test pointed out significant decreases in the amended soils over time, from time 1 to time 2 (Table 4).

As regards P_{tot} and P_{avail}, the two-way nested ANOVAs revealed a significant impact of the OMW disposal at both sites (Table 3). In site A, at time 1 both P forms were statistically higher in the OMW treated soil than in the control, whereas six months after only P_{tot} was

Table 4
Soil chemical parameters of two olive mills (site A and site B) at different sampling times after olive mill wastewater (OMW) addition, five days and six months, in comparison with controls (unamended soils).

Soil parameters[a]	Site A[b]		Site B	
	OMW	Control	OMW	Control
Five days[c]				
pH	7.43 ± 0.03[d] aA	7.97 ± 0.09 bB	7.33 ± 0.05 A	7.33 ± 0.09 A
EC (mS cm^{-1})	0.80 ± 0.06 bB	0.30 ± 0.03 a	0.67 ± 0.05 bB	0.22 ± 0.03 a
K_{exch} (mg kg^{-1})	2140.33 ± 168.16 bB	221.67 ± 25.06 a	1634.00 ± 191.92 bB	389.00 ± 44.69 a
N_{tot}(g kg^{-1})	5.77 ± 0.81 bB	1.53 ± 0.16 a	1.21 ± 0.06 B	1.14 ± 0.06
NO_3^- (mg kg^{-1})	5.67 ± 0.33 B	5.00 ± 2.52	7.90 ± 1.40 bB	3.10 ± 1.42 a
NH_4^+ (mg kg^{-1})	20.00 ± 1.53 bB	9.00 ± 1.00 aB	14.78 ± 4.36 b	0.45 ± 0.14 aA
SOC (g kg^{-1})	59.08 ± 18.64 bB	15.11 ± 3.13 a	10.49 ± 0.63 B	9.67 ± 0.59
P_{tot}. (g kg^{-1})	2.83 ± 0.43 b	1.50 ± 0.05 a	1.43 ± 0.11 B	1.26 ± 0.07
P_{avail} (mg kg^{-1})	257.00 ± 22.37 bB	9.67 ± 2.19 a	75.00 ± 6.10 B	105.50 ± 16.11
Soluble phenols (mg coumaric acid kg^{-1})	36.67 ± 7.06 bB	2.67 ± 0.67 a	67.75 ± 16.91 bB	5.75 ± 1.11 a
Six months				
pH	8.15 ± 0.03 bB	7.78 ± 0.06 aA	7.75 ± 0.14 B	7.77 ± 0.13 B
EC (mS cm^{-1})	0.27 ± 0.01 aA	0.35 ± 0.03 b	0.15 ± 0.01 aA	0.25 ± 0.02 b
K_{exch} (mg kg^{-1})	304.50 ± 30.73 bA	227.25 ± 9.55 a	373.67 ± 15.08 bA	285.33 ± 22.26 a
N_{tot}.(g kg^{-1})	1.44 ± 0.07 A	1.37 ± 0.06	1.00 ± 0.06 A	1.17 ± 0.08
NO_3^- (mg kg^{-1})	1.68 ± 0.45 A	2.33 ± 0.76	2.33 ± 0.88 A	1.81 ± 0.51
NH_4^+ (mg kg^{-1})	0.70 ± 0.50 A	0.95 ± 0.75 A	10.67 ± 1.67	9.33 ± 0.33 B
SOC (g kg^{-1})	11.41 ± 0.39 A	11.21 ± 0.48	8.31 ± 0.46 A	10.04 ± 0.57
P_{tot} (g kg^{-1})	1.83 ± 0.00 b	1.43 ± 0.06 a	0.87 ± 0.38 A	1.73 ± 0.31
P_{avail} (mg kg^{-1})	19.75 ± 1.89 A	12.25 ± 3.12	40.00 ± 4.04 aA	122.33 ± 6.44 b
Soluble phenols (mg coumaric acid kg^{-1})	2.25 ± 0.25 A	4.00 ± 0.71	6.00 ± 0.00 A	6.33 ± 0.33

[a] EC: electrical conductivity; K_{exch}: exchangeable potassium; N_{tot}: total nitrogen; NO_3^-: nitrate; NH_4^+: ammonium; SOC: soil organic carbon; P_{tot}: total phosphorus; P_{avail}: available phosphorus.
[b] Site A: Caprona, Pisa, Italy; Site B: Braccagni, Grosseto, Italy.
[c] Means: SE of at least three replicate field plots per treatment. For each site values not followed by the same small letter and by the same capital letter are significantly different between OMW treatments within time and within OMW treatments between time, respectively, according to the *t*-test ($P < 0.05$). Q3

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significantly higher in comparison with the untreated plots. In site B, at time 2 the treated areas showed values of P_{avail} lower than in the control. In addition, the *t*-test pointed out in the OMW plots of both sites significant reductions of P_{avail} between spreading time (Table 4).

Finally, at both sites, the OMW treatment produced a significant impact on soil soluble phenols (Table 3). In detail, at spreading time, soluble phenols in the amended soils were 13-fold higher than the values in the controls, although this concentration decreased significantly at time 2 to values similar to the unamended plots (Table 4).

3.2. Biochemical parameters

The mixed model two-way nested ANOVAs pointed out similar patterns of the biochemical parameters as affected by the OMW application (Table 3). Actually, MBC, SR, microbial and metabolic quotients, used for assessing soil biochemical changes, were significantly influenced by the OMW treatment (Table 3).

Significant short-term changes of MBC were observed in the amended soils as compared with the controls, while no long-term changes were detected (Table 5). As regards the short-term assessment, MBC decreased at site A and site B about four-fold and eight-fold, respectively, in the soils amended by OMW in comparison with the controls. In both sites, the microbial quotient decreased 14- and 10-fold, respectively, after the OMW disposal (Table 5). With regard to long-term assessment, the *t*-test over time highlighted at both amended sites the ability of soil to restore its normal MBC (Table 5). Actually, significant increases of both MBC and Cmic/Corg were recorded in the amended soils at time 2 in comparison with time 1. By contrast, no changes were revealed in the MBC of the control soils of site B from spreading (November) to time 2 (May) and in the Cmic/Corg at both control sites between the two sampling times (Table 5). Interestingly, at site A, seasonality was observed in the MBC, as it decreased also in the control sites from spreading (May) to time 2 (November).

The microbial activity, evaluated by SR and qCO_2, was significantly higher at time 1 at both sites, except for the SR of site B, while no changes were detected in the amended *vs* the unamended plots at time 2 (Table 5). In detail just after spreading, the qCO_2 of both OMW treated soils was six-fold higher the values observed in the controls, while SR at site A was 87% higher than the control (Table 5). The *t*-test over time pointed out in both the amended soils a strong decrease from spreading to time 2 of both biochemical indicators to values similar to the controls (Table 5).

3.3. AM fungal measurements

The microscopical assessment allowed the visualisation of the AM fungal root colonisation, arbuscules and vesicles within the roots of *L. perenne* occurring in the amended and unamened plots. A differential behaviour of the AM fungi colonising the roots of *L. perenne*, occurring in the amended soils in comparison with the controls, was observed at both sites. The mixed model two-way nested ANOVAs pointed out at site A significant impact of the application of OMW on AM fungal root colonisation, arbuscules and vesicles (Table 3). In the amended soils of site A we detected a decrease of the AM fungal root colonisation and a significant increase of the two other characteristic structures of the intraradical AM fungal system (Tables 3, 5). In detail, root colonisation was 43% and 34% lower in the treated soil in comparison with the control, at time 1 and 2, respectively (Table 5). As regards arbuscules and vesicles, within the roots of *L. perenne* in the amended soil we observed at spreading values of arbuscules 49% higher than in the control and at time 2 values of vesicles 40% higher in comparison with the untreated plots (Table 5).

At site B, a significant impact of the OMW application was detected only on arbuscules (Table 3). Actually, while arbuscules increased within the roots of *L. perenne* in the amended soil, root colonisation and vesicles did not change, although root colonisation significantly varied over time (Tables 3, 5). In detail, consistently with what observed in site A at spreading, a large AM fungal root colonisation decrease (61%) along with a strong increase of arbuscules (86%) was detected in the treated soil in comparison with the control (Table 5). In addition, at time 2, only arbuscules were significant higher in the amended soil than in the unamended one (Table 5).

Table 5
Soil biochemical and arbuscular mycorrhizal (AM) fungal parameters of two olive mills (site A and site B) at different sampling times after olive mill wastewater (OMW) addition, five days and six months, in comparison with controls (unamended soils). Q4

Soil parameters[a]	Site A[b]		Site B	
	OMW	Control	OMW	Control
Five days				
Biochemical				
Microbial biomass carbon (mg C kg^{-1} soil)	53.33 ± 6.77[c] aA	217.33 ± 17.93 bB	31.50 ± 3.77 aA	275.00 ± 6.81 b
Soil respiration (mg CO_2–C kg^{-1} soil)	246.40 ± 8.10 bB	131.71 ± 4.97 a	102.88 ± 35.29 a	216.79 ± 22.47 b
Cmic/Corg (%)	0.11 ± 0.40 aA	1.52 ± 0.26 b	0.30 ± 0.04 aA	2.87 ± 0.18 b
qCO_2 (mg CO_2–C mg $Cmic^{-1}$ d^{-1})	4.83 ± 0.83 bB	0.61 ± 0.02 a	3.04 ± 0.86 bB	0.79 ± 0.08 a
AM fungi				
Root colonisation (%)	17.74 ± 0.59 a	31.30 ± 2.25 bB	2.56 ± 1.01 aA	6.57 ± 1.23 bA
Arbuscules (%)	51.33 ± 4.18 b	34.33 ± 4.09 a	50.25 ± 2.06 b	27.00 ± 1.83 a
Vesicles (%)	10.67 ± 1.20 A	11.33 ± 0.88	12.50 ± 1.71	9.75 ± 1.18
Six months				
Biochemical				
Microbial biomass carbon (mg C kg^{-1} soil)	144.50 ± 27.87 B	147.45 ± 28.00 A	191.87 ± 25.66 B	242.66 ± 38.48
Soil respiration (mg CO_2–C kg^{-1} soil)	104.83 ± 28.65 A	162.64 ± 30.05	143.55 ± 3.80	150.79 ± 20.69
Cmic/Corg (%)	1.27 ± 0.14 B	1.31 ± 0.10	2.31 ± 0.27 B	1.82 ± 0.55
qCO_2 (mg CO_2–C mg $Cmic^{-1}$ d^{-1})	0.74 ± 0.19 A	1.26 ± 0.33	0.77 ± 0.08 A	0.62 ± 0.02
AM fungi				
Root colonisation (%)	15.67 ± 2.10 a	23.69 ± 1.72 bA	31.47 ± 4.54 B	22.84 ± 7.19 B
Arbuscules (%)	54.50 ± 5.56	44.25 ± 3.50	48.00 ± 4.58 b	18.00 ± 3.61 a
Vesicles (%)	18.25 ± 2.22 bB	13.00 ± 1.83 a	11.67 ± 2.03	9.33 ± 1.20

[a] MBC: microbial biomass carbon; SR: soil respiration; Cmic/Corg: microbial carbon/soil organic carbon ratio; qCO_2: metabolic quotient, SR/MBC.
[b] Site A: Caprona, Pisa, Italy; Site B: Braccagni, Grosseto, Italy.
[c] Means ± SE of at least three replicate field plots per treatment. For each site values not followed by the small and capital letter are significantly different between OMW treatments within time and within treatments between time, respectively, according to the *t*-test ($P < 0.05$).

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The *t*-test over time pointed out, as regard site B, significant increases of the AM fungal root colonisation both in the treated soil and in the control from November to May and, as regards site A, a significant decrease in the control from May to November (Table 5). Moreover, in the treated soils of site A, this test revealed a significant increase of the percentage of vesicles from time 1 (May) to time 2 (November) after spreading (Table 5).

3.4. Short- and long-term main patterns of chemical, biochemical and AMF as affected by OMW spreading

PERMANOVA analyses (Fig. 1) showed that in both studied areas soil chemical, biochemical and AM fungal parameter assemblages clearly differed between amended and unamended plots, while time did not show any significant impact (Table 6). In the PCO analyses the first two principal coordinates explained 87.9% and 7.3% of the variance at site A, while at site B 79.1% and 11.3% (Fig. 1). With respect to these two principal coordinates, at both sites, amended soil at time 1 can be attributed to the major cluster with respect to the other treatments (unamended soil at time 1 and both soils, amended and unamended, at time 2; Fig. 1). In addition, at both sites, the soil parameters of the unamended areas, evaluated after short- and long-term periods, seems more similar to each other with respect to the amended areas at time 2.

In detail, the arrows representing the majority of the chemical parameters monitored in the present study point to the soil at time 1, clearly showing their higher values compared with the other treatments. With regard to biochemical and AM fungal parameters, the patterns of the arrows are less consistent between the two sites. Actually, at site A the arrows representing MBC and AM fungal colonisation show that their values are higher in the unamended soil at time 1 (May), and the arrows representing SR that its value is

Table 6
Results of PERMANOVA analyses on soil chemical, biochemical and arbuscular mycorrhizal (AM) fungal parameters between olive mill wastewater (OMW) treatments (amended soil *vs* control) in two different olive mills (site A and site B) and time, five days and six months after OMW addition.

Source of variation[a]	df	SS	MS	Pseudo-*F*	*P* (perm)
Site A					
Time[b]	1	299.590	299.590	1.392	0.167
OMW	2	439.180	219.590	33.173	0.001
Residual	10	66.199	6.619		
Total	13	804.960			
Site B					
Time	1	171.200	171.200	0.847	0.658
OMW	2	412.390	206.190	22.042	0.001
Residual	10	93.547	9.355		
Total	13	677			

[a] Source of variation, df (degrees of freedom), SS (sum of squares), MS (mean squares) Pseudo-*F* (*F* value by permutation) and *P* (perm) (*P* value by permutation) are given.
[b] Mixed model two-way nested design PERMANOVA: time, fixed factor; OMW: random factor nested within time; replicate field plots were at least three per treatment.

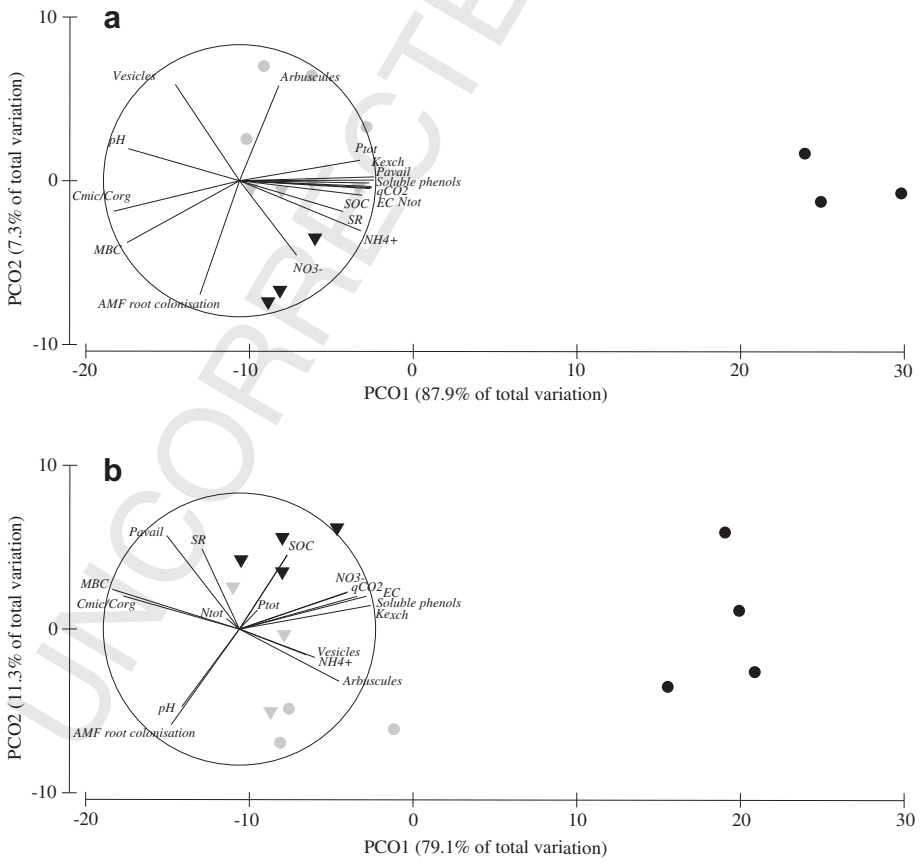

Fig. 1. Principal coordinate analyses (PCO) computed on Bray–Curtis coefficient of similarities between soil samples on the basis of chemical, biochemical and arbuscular mychorrizal (AM) fungal parameters which were previously square root transformed. (a) Olive mill located in Caprona, Pisa, Italy (site A): olive mill wastewater (OMW) amended soils and their corresponding controls (unamended soils) five days and six months after spreading; (b) Olive mill located in Braccagni, Grosseto, Italy (site B): OMW amended soils and their corresponding controls five days and six months after spreading. PCO1 and PCO2 are the first and the second principal coordinates, respectively. The proportion of variance explained by the principal coordinates is given in parentheses. Black and grey represent five days and six months after OMW spreading, respectively. Circle and upper triangle symbols represent OMW amended and unamended soils, respectively.

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higher in the amended soil at time 1 (Fig. 1a). By contrast, at site B the arrows representing MBC and AM fungal colonisation show that their values are higher in amended and unamended soil at time 2 (May), and the arrows representing SR that its value is higher in unamended soil at both spreading times (Fig. 1b).

Furthermore, at site A the diagram points out interesting correlations among EC, K_{exch}, N_{tot}, NH_4^+, SOC, P_{tot}, P_{avail}, soluble phenols, SR and qCO_2, while at site B among different parameters: (i) EC, K_{exch}, NO_3^-, soluble phenols and qCO_2; (ii) NH_4^+, arbuscules and vesicles; (iii) N_{tot}, P_{avail} and SR; (iii) pH and AM fungal root colonisation.

4. Discussion

In this work for the first time we assessed the short- and long-term effects of long-lasting repeated OMW field applications on soil chemical and biological parameters. PCO analyses together with PERMANOVAs showed that OMW spreading: (i) affected soil chemical properties, microbial biomass carbon, soil respiration, AM fungal root colonisation, arbuscules and vesicles in the short-term period in comparison with control, whereas it did not determine long-term residual effects irrespective of the application times; (ii) decreased AM fungal root colonisation both after autumn and spring OMW applications; (iii) improved arbuscule occurrence in the short- and long-term for both disposal times.

4.1. Chemical parameters

Several studies have been performed on the effects of organic amendments and OMW land spreading, showing benefits on soil fertility and productivity by ameliorating the nutritional and biological equilibrium in the soil–plant system (Levi-Minzi et al., 1992; Cabrera et al., 1996; Paredes et al., 1999; Alianello, 2001; Saadi et al., 2007; Kapellakis et al., 2008; Chartzoulakis et al., 2010).

Most of these studies showed that, while the OMW addition caused short and sudden decreases of soil pH (Alianello, 2001; Di Serio et al., 2008; Arienzo et al., 2009; Kavvadias et al., 2010), no negative effects were observed over time when appropriate doses were utilised (Levi-Minzi et al., 1992; Cabrera et al., 1996; Chartzoulakis et al., 2010). Here, consistently with what was previously observed, in site A, five days after OMW spreading, we detected a significant lower soil pH in the treated soils than in the control. Nevertheless this reduction is low, since soil pH value shifted from moderately to slightly alkaline (7.97 *vs* 7.43), according to the USDA classification (Soil Survey Division Staff, 1993a). In addition, soil pH increased over time in treated soils, whereas decreased in the controls. This behaviour may be explained by the pH seasonal variability and by the breakdown caused by MBC (Soil Survey Division Staff, 1993b; Thomas, 1996). By contrast, in site B the pH of the amended soils did not change in comparison with the control at both sampling times. This pattern was reported also by Chartzoulakis et al. (2010) and can be related to the quality of the OMW applied, to the time of spreading and to the soil properties.

Here, at both sites EC was significantly affected by the application of OMW, showing increases five days after spreading and decreases six months later in the treated soils compared with the controls. Nevertheless, in both sites, EC values remained below the salinity threshold (4 mS cm^{-1}). Our findings were consistent with previous works, reporting EC increases during the irrigation times and decreases in between them (Cabrera et al., 1996; Mekki et al., 2007; Sierra et al., 2007; Chartzoulakis et al., 2010; Kavvadias et al., 2010; Moraetis et al., 2011).

Here, soil K_{exch} was higher in the treated soils of both sites in comparison with the controls irrespective of time after disposal and spreading period. These increases are directly caused by the high content of K in the OMW and are in agreement with what has been previously observed by several authors (Mechri et al., 2007; Di Serio et al., 2008; Chartzoulakis et al., 2010; Kavvadias et al., 2010). These K increases are beneficial for crop productivity and health according to the different plant requests and uptake efficiency (Arienzo et al., 2009) and can have ecological and economical advantages avoiding or reducing the use of K fertilisers (Di Serio et al., 2008).

SOC did not change after OMW application, except at time 1 in site A. These results are consistent with those reported over a year by Gamba et al. (2005) in similar climatic conditions. Nevertheless, some studies showed that SOC increases were temporary, since the organic matter brought by OMW is rapidly mineralised by soil microorganisms (Alianello, 2001; Sierra et al., 2007; Piotrowska et al., 2006, 2011; Mechri et al., 2007; Di Serio et al., 2008). In this regard, the short-term sharp SOC increase detected in site A can be explained by the composition of the OMW, which showed values of BOD and COD higher than those spread in site B.

Similarly to SOC, long-lasting repeated OMW spreading did not cause significant increases in N_{tot}, except for five days after disposal in site A. In agreement, while increases were observed in the short-term period by several authors (Mechri et al., 2007; Sierra et al., 2007), decreases were reported over time by Sierra et al. (2007). As regards mineralised-N, positive effects were observed in NO_3^- and in NH_4^+ five days after disposal, although at site A NO_3^- increases were not significant. Moreover, at both sites, the concentration of these available mineral forms declined to levels similar to the corresponding unamended soils six months after OMW spreading. Our data are consistent with the results of López-Piñeiro et al. (2006), showing field increases of NO_3^- due to long-lasting repeated OMW spreading, that seem to be linked to the NH_4^+ increases (Stvenson and Cole, 1999). The mineralised-N forms patterns might be related to soil chemical–physical properties, different time of observation and climate, which can influence the biological activity. In addition, these findings suggested no immobilisation of mineral N during the mineralisation of the easy degradable organic matter applied by OMW and no negative impacts on nitrifying bacteria. In any case, our concentrations are remarkably low in comparison with those found in most organically amended soils (Balík et al., 2003; Ros et al., 2006; Sadej and Przekwas, 2008).

Increases of P_{tot} and P_{avail} were observed in site A five days after OMW spreading, while no changes were shown in site B. These differences can be due to the quality of OMW applied. In fact, the OMW used in site A were more acidic and had 25% and 59% higher P_{tot} and organic load than those used in site B. Similar results were found by several authors studying soil P changes in the short-term due to OMW applications (Cabrera et al., 1996; Alianello, 2001; Piotrowska et al., 2006; Sierra et al., 2007; Di Serio et al., 2008). As regards long-term impact, our data are in agreement with most of the studies generally observing no changes in the amended sites (Alianello, 2001; Piotrowska et al., 2006; Kavvadias et al., 2010).

The high content of polyphenols in the OMW strongly affected their occurrence in the soil at both sites, although such increases were not stable over time. Several studies highlighted similar polyphenol short-term trends and pointed out their negative impact on microbial biomass and their phytotoxic effects due to flavonoids (Alianello, 2001; Saadi et al., 2007; Chartzoulakis et al., 2010). Moreover, polyphenol decreases over time due to decomposition or incorporation into the humic fraction of the SOC are shown by several authors (Sierra et al., 2001; Gamba et al., 2005; Saadi et al., 2007; Chartzoulakis et al., 2010).

Overall, the severe reduction from time 1 to time 2 of the concentration of K_{exch} might be caused only by a partial assimilation of the plants and by a significant leaching phenomena. This hypothesis could be confirmed by the similar trend of soluble

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phenols, EC, and $P_{avail.}$ In this view, additional data such as rainfall, plant roots barrier, soil porosity should be considered for approaching eventual ecological risks in the surface and groundwater.

4.2. Biochemical parameters

At both sites, significant reductions in MBC were observed, while a significant increase in SR was found only in site A. By contrast, over time no negative impacts on these key indicators of soil fertility were detected, confirming the short turnover of MBC (Jenkinson and Ladd, 1981).

As regards MBC, our data are in contrast with those reported by other authors monitoring short-term MBC changes following OMW incorporation, although such studies assessed the impact of single OMW application. Strong increases of MBC were found by Tardioli et al. (1997) and by Piotrowska et al. (2011) at laboratory scale, and by Gamba et al. (2005) in the field. However, polyphenols, which are abundant in OMW, are shown to act as growth inhibitors against soil microbes by affecting moulds, yeasts, actinomycetes, ammonifiers, nitrogen aerobes and cellulolitics, and by reducing their capacity of degradation (Alianello, 2001; Saadi et al., 2007).

Moreover, SR short-term increases were largely reported in several laboratory and field studies, measuring the CO_2 produced by microbes in response to OMW application (Mekki et al., 2006a,b; Di Serio et al., 2008). Recently, Piotrowska et al. (2011) detected over a period of a month after the amendment, beneficial increases of MBC due to organic matter inputs and also of SR, confirming the high microbial stress caused by OMW spreading. Such "priming effect" shown by SR can be correlated with the ability of microorganisms to decompose different organic substrates and seem to be induced by the labile part of the organic matter applied by OMW (Di Serio et al., 2008).

Here, microbial stress due to soil disturbance was assessed by the metabolic quotient (qCO_2) and by Cmic/Corg. In detail, the less qCO_2 is assessed, the more microbes are efficient (Kutsch et al., 2009; Pellegrino et al., 2011). In this regard, the increase of qCO_2 observed here just after land spreading in both treated sites is in accordance with what Piotrowska et al. (2011) revealed, while the Cmic/Corg patterns are consistent with the strong and short reduction of MBC at both sites.

4.3. AMF measurements

So far, to our knowledge, no information is available on the AM fungi colonising roots after field long-lasting repeated OMW spreading using morphological visualisation and assessment. Recently, while Mechri et al. (2008) evaluated the effect on AMF of a single OMW application in the field one year after treatment using the soil fatty acid methyl ester (FAME), Ipsilantis et al. (2009) assessed in microcosms the short-term impact of OMW on AM fungal root colonisation, hyphal length and community pattern of native pre-propagated/inoculated AMF.

Here, a significant decrease of the AM fungal root colonisation together with an increase of arbuscules were consistently observed five days after OMW spreading at sites A and B, while six months after OMW disposal such parameters were more variable, with colonisation decreases in site A and arbuscules increases at site B. The significant reductions of AM fungal root colonisation assessed at time 1 and 2 for site A and at time 1 for site B are consistent with that observed by Ipsilantis et al. (2009) in *Vicia faba* root colonisation just after spreading and by Mechri et al. (2008) in the soil fatty acid signature one year after OMW spreading. Moreover, while no effects were detected on AM fungal root colonisation of *Retama sphaerocarpa* (Caravaca et al., 2006), lower rates of colonisation were also observed in tomato, lucerne, soybean and lettuce after dry olive mill residue application (Martín et al., 2002; Sampedro et al., 2009). Such AM fungal root colonisation reductions can be related to the chemical changes of pH, EC, K_{exch}, N, SOC, P and soluble phenols observed in soil after OMW disposal. In fact, it is well known that the distribution of some AM fungal species is correlated with soil pH (Abbott and Robson, 1977; Porter et al., 1987) and that the development and functioning of AMF, as well as the germination and hyphal growth of some species, are affected by its changes (Hepper, 1984; Abbott and Robson, 1985; Porter et al., 1987; Clark, 1997). Moreover, AM fungal root colonisation was shown to generally decline as EC, K_{exch}, N, SOC and P increased (Hays et al., 1982; Abbott and Robson, 1991). As regards phenols, recently, Piotrowski et al. (2008), observing significant reductions on *Sorghum sudanese* AM fungal root colonisation, highlighting the potential mechanisms of inhibition of such compounds on the establishment of AM symbioses.

As regards site B, the non residual effects observed in the present study may be related to the times of application, that seem to be a key factor for reducing the long-term impact of OMW on these fundamental microbes, and to the quality of the OMW utilised. In this regard, the OMW pH of site B was higher than in site A, while K, N, P and the OM contents were largely lower.

Unexpectedly, the percentage of AM fungal root colonisation showed by *L. perenne* grown in November just after OMW spreading in the amended and unamended plots of site B was strongly lower than that detected at site A in May (five days after spreading) and also in November (six months after disposal). Such a difference can be explained not only by the OMW disposal, but also by the seasonal patterns observed in mycorrhizal occurrence, morphology and diversity (Sanders and Fitter, 1992; Daniell et al., 2001; Dumbrell et al., 2011) and by plant community through host-preference/specificity (Bever et al., 1996; Vandenkoornhuyse et al., 2002; Hausmann and Hawkes, 2009). In fact, in site B the high abundance of the non-host *B. nigra* (Harley and Harley, 1987) may explain the low AM fungal root colonisation.

5. Conclusions

The present study showed that, despite long-lasting repeated OMW land spreading, no residual effects or negative trends are observed in soil chemical and biochemical parameters, even though there are relevant effects in the short-term. Therefore, these effects do not persist and should be considered negligible after a suitable waiting period. As regards AMF, significant decreases of AM fungal root colonisation at both sampling times need to be taken into consideration, although the observed increases of arbuscules might be considered as a marker of good functionality. In the controls most of the monitored parameters appear stable at both sites except for pH and NH_4^+ that varied following the season (November or May) instead of the sampling time (five days and six months), while in the treated plots we observed temporal variations that tend to realign the conditions of the soil to the original ones. This temporal behaviour is clearly evident in the soil polyphenol trend, which may be considered the key indicator of OMW land spreading risk. As regards OMW management strategies, the effects on OMW quality, AM fungal root colonisation and most of the soil chemical parameters were significant, whereas no effect on the soil biochemical properties were observed. Therefore, we can argue that at controlled doses (80 m^3 ha^{-1}) and using suitable spreading methods (soil mixing, regularity of distribution, water content in soil, runoff events avoided), OMW can be utilised as organic amendment in agriculture because of no negative long-term residual effects. Nevertheless, to improve the use of these waters in agriculture further studies are needed on their

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environmental risks to surface and groundwater contamination, soil microorganism, microarthropods and/or earthworms.

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