Journal of Environmental Science and Health, Part B: Pesticides, Food Contaminants, and Agricultural Wastes
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/lesb20

Short-term impact of olive mill wastewater (OMWW) applications on the physico-chemical and microbiological soil properties of an olive grove in Argentina

Pierluigi Pierantozzi [a], Mariela Torres [b], Romina Verdenelli [a], María Basanta [c], Damián M. Maestri [a] & José M. Meriles [a]

[a] Instituto Multidisciplinario de Biología Vegetal (IMBIV, CONICET-UNC), Instituto de Ciencia y Tecnología de los Alimentos (ICTA), Físicas y Naturales (FCEFyN-UNC), Córdoba, Argentina

[b] Estación Experimental Agropecuaria San Juan (EEA INTA San Juan), Consejo Nacional de Investigaciones Científicas y Técnicas, San Juan, Argentina

[c] Estación Experimental Agropecuaria (EEA - INTA), Córdoba, Manfredi, Argentina

To cite this article: Pierluigi Pierantozzi , Mariela Torres , Romina Verdenelli , María Basanta , Damián M. Maestri & José M. Meriles (2013): Short-term impact of olive mill wastewater (OMWW) applications on the physico-chemical and microbiological soil properties of an olive grove in Argentina, Journal of Environmental Science and Health, Part B: Pesticides, Food Contaminants, and Agricultural Wastes, 48:5, 393-401

To link to this article: http://dx.doi.org/10.1080/03601234.2013.742398

Journal of Environmental Science and Health, Part B (2013) **48**, 393–401

ISSN: 0360-1234 (Print); 1532-4109 (Online)
DOI: 10.1080/03601234.2013.742398

Short-term impact of olive mill wastewater (OMWW) applications on the physico-chemical and microbiological soil properties of an olive grove in Argentina

PIERLUIGI PIERANTOZZI[1], MARIELA TORRES[2], ROMINA VERDENELLI[1], MARÍA BASANTA[3], DAMIÁN M. MAESTRI[1] and JOSÉ M. MERILES[1]

[1]*Instituto Multidisciplinario de Biología Vegetal (IMBIV, CONICET-UNC), Instituto de Ciencia y Tecnología de los Alimentos (ICTA), Físicas y Naturales (FCEFyN-UNC), Córdoba, Argentina*
[2]*Estación Experimental Agropecuaria San Juan (EEA INTA San Juan), Consejo Nacional de Investigaciones Científicas y Técnicas, San Juan, Argentina*
[3]*Estación Experimental Agropecuaria (EEA - INTA), Córdoba, Manfredi, Argentina*

The purpose of this work was to investigate the effects of spreading olive oil mill wastewater (OMWW) on soil biochemical parameters and olive production in an organically managed olive orchard. The experiment was carried out with three different doses of OMWW (80, 160 and 500 m^3 ha^{-1}) and a control (untreated soil). Three samplings were done at 10, 30 and 90 days after the administration of the byproduct. OMWW application differentially modified the biochemical properties of the soil analyzed. Organic matter, organic carbon, total nitrogen and extractable phosphorus soil contents increased proportionally with each increasing dose. The values of these parameters decreased gradually with time. Total microbial activity was altered and the OMWW 500 m^3 ha^{-1} treatment proved to be the most active when compared with the other applied doses. OMWW agricultural application also modified the structure of soil microbial communities, particularly affecting Gram positive and negative bacteria, while fungal biomass did not show consistent changes. Although there was a salinity increase in the treated soil, especially at the highest dose, the productive parameters analyzed (fruit and oil $tree^{-1}$) were not affected. In light of the obtained results, we consider that low dose of OMWW could be considered an alternative farming practice for semiarid regions.

Keywords: *Olea europaea* L., olive oil mill wastewater, land spreading, soil microbiological properties, microbial community structure, phospholipids fatty acid.

Introduction

During the last fifteen years, the olive crop in Argentina has grown to approximately 100,000 ha. More than 90% of this production takes place on irrigated land. The country thus has the 13 largest cultivated surface dedicated to olive crops in the world.[1] Productive areas in Córdoba are primarily located in the Northwestern county of Cruz del Eje, and, to a lesser extent, in Ischilín and Traslasierra Valley, near the town of Villa Dolores in the counties departments of Cruz del Eje and San Alberto. The olive groves are traditional low-density plantations, with approximately 100 plants ha^{-1}.[2]

Seventy percent of the local olive production is used for oil manufacturing, which generates by-products that can pose serious environmental and storage problems, especially in light of the large volume of production. In Córdoba, 70% of the olive oil comes from the press process and the three-phase centrifuge system, which produce a considerable amount of olive oil mill wastewater (OMWW). A medium size oil mill will process 10 to 20 Tn olives per day, turning out 0.75 m^3 Tn^{-1} of OMWW. Hence, daily volume of OMWW can reach well over 8 m^3.

OMWW is made of water from the fruit itself, combined with the washing and processing water plus pulp soft tissues and oil, which forms a stable emulsion.

OMWW is highly toxic because of its high content of phenolic compounds and organic matter. It has very high biological oxygen demand (BOD_5) and the chemical oxygen demand (COD) that go beyond the maximum acceptable limit for discharge into reservoirs according to Provincial

Address correspondence to Pierluigi Pierantozzi, Instituto Multidisciplinario de Biología Vegetal (IMBIV, CONICET-UNC), Instituto de Ciencia y Tecnología de los Alimentos (ICTA), Facultad de Ciencias Exactas, Físicas y Naturales (FCEFyN-UNC), Av. Vélez Sarsfield 1611, X5016GCA, Córdoba, Argentina; E-mail: p.pierantozzi@hotmail.it
Received July 4, 2012.

Law 415/99 (maximum values 100 g L^{-1} and 200 g L^{-1}, respectively).[3–10] Additionally, depurification is extremely difficult, even when applying the standard urban sewage active sludge methods.

In many olive-growing countries, including even the most advanced, the problem of disposing and treating this biomass has not yet been solved, Thus far, only temporary solutions have been adopted to solve this problem. The use of storage basins developed in some countries to evacuate vegetable water has not been successful because of the musty smell and bad management of the dried remainders. The use of vegetable water as fertilizer and an organic amendment for agricultural lands has been proposed as a rational technique to exploit the residual liquid effluent. OMWW could be used as an alternative source of fertilizer-irrigation systems in arid and semiarid regions, such as those found in Argentina, because of its high fertilizing value, availability in the dry winter season, and low risk of contamination as the area's aquifer lies at a depth of more than 100 m.

There are several studies which have examined the impact of OMWW on the chemical, biochemical and biological properties of soil. In most studies, the effects of the application of OMWW on agricultural soils occur mainly in the first period after the amendment.[11–14]

The main objective of this work was to investigate the effects of spreading OMWW on soil biochemical parameters and olive production in an organically managed olive orchard.

Materials and methods

Description of the study site, soil analysis and OMWW

The study was carried out in a commercial olive plantation (*Olea europaea* L.) cv. *Manzanilla* in Las Playas, near the town of Cruz del Eje, Córdoba, Argentina. Seventy-year-old "Manzanilla" olive trees, spaced at 10 × 10 m, were selected for the assay. The trees were vase-trained and managed with superficial irrigation through the basin, according to the schedule typically used in the area.

The Cruz del Eje production zone is in the NW corner of the province, between 30°–31° South and 64°–65° W, at 450 masl. It is part of the phytogeographical region of Chaqueño forest.[15]

The climate of the region is dry moderate with alternating warm winds from the north and cool winds from the south. January is the hottest month while July is the coldest. Winter is not harsh, with only a few days of frost. Summer is the rainy season and the total spring-summer rainfall is 420 mm. In autumn-winter the precipitation reaches approximately 130 mm (Fig. 1).

Before the implementation of the different treatments, two soil surveys were carried out on neighboring ground with plants of a similar size and age as those selected for the experiment. This analysis established that the soil within the irrigated area was 60–65 cm deep and corresponded to a typical Haplustoll with sandy loam in the superficial horizon and silt loam further down, poor in nitrogen but rich in calcium and potassium (Table 1). In most cases, with a pH higher than 7, the danger of salinization, with good management, is low.

OMWW or "alpechín" was collected from a traditional discontinuous press system at an oil factory, during the 2007–2008 olive production period. Sampling was performed according to the recommended standard methods.[16] Three samples (approximately 5 L each) were taken, placed in dark containers and kept at 4°C in Styrofoam boxes before being carried to the laboratory.

The time between sample collection and physicochemical analysis did not exceed 10 h. OMWW was tested for COD (g O_2 L^{-1}), BOD_5 (g O_2 L^{-1}), electrical conductivity (EC, dS m^{-1}) and pH using standard methods.[16] Phenolic compounds were extracted using the Macheix et al.

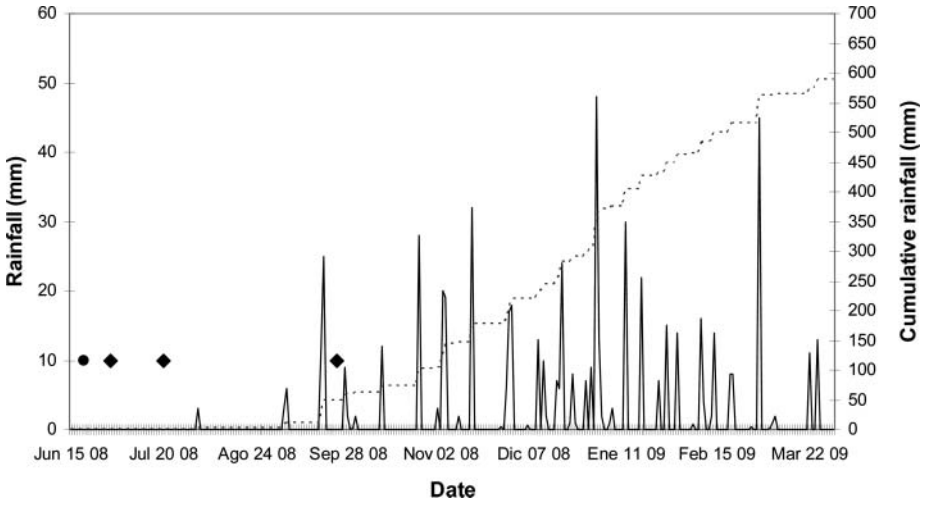

Fig. 1. Rainfall events (solid line) and cumulative rainfall (dashed line) in the study area, during the 2008/09 crop season. Circle denotes OMWW application. Rhombuses denote soil sampling events.

Table 1. Physical and chemical characteristics of the soil horizons from the olive growing area at Cruz del Eje location (Córdoba province, Argentina).

	Horizons			
Soil properties	*A1 y A3*	*Bw*	*BC*	*C*
Depth	0–25	30–40	50–60	70–80
Organic matter content (%)	2.65 ± 0.11	1.68 ± 0.21	1.02 ± 0.07	0.66 ± 0.05
Kjeldahl nitrogen (%)	0.15 ± 0.02	0.11 ± 0.02	0.09 ± 0.01	0.07 ± 0.01
Extractable phosphorus (ppm)	22.0 ± 1.40	17.0 ± 0.80	6.00 ± 0.80	3.00 ± 0.40
Very coarse sand (1–2 mm) (%)	7.45 ± 0.31	6.69 ± 0.22	—-	4.05 ± 0.35
Coarse sand (500–1000μ) (%)	8.21 ± 0.31	7.51 ± 0.31	—-	5.02 ± 0.21
Medium sand (250–500μ) (%)	15.0 ± 0.06	11.4 ± 0.28	—-	13.5 ± 0.22
Sand (100–250μ) (%)	18.0 ± 0.45	13.0 ± 0.22	—-	12.8 ± 0.28
Very fine sand (50–100μ) (%)	15.0 ± 0.11	11.2 ± 0.35	—-	12.7 ± 0.45
Coarse silt (20–50μ) (%)	18.0 ± 0.19	25.0 ± 0.55	—-	43.7 ± 0.61
Fine silt (2–20μ) (%)	9.00 ± 0.07	10.2 ± 0.41	—-	6.45 ± 0.72
Clay (<2μ) (%)	10.0 ± 0.85	15.5 ± 0.07	—-	2.14 ± 0.15
pH	7.86 ± 0.32	7.93 ± 0.24	8.00 ± 0.22	8.55 ± 0.14
Electrical conductivity (dS m^{-1})	1.50 ± 0.05	0.90 ± 0.02	0.80 ± 0.01	0.80 ± 0.03

Mean values ± standard deviation values.

method.[17] Total phenolic content (TPC) was measured using Folin–Ciocalteu reagent according to Torres et al.[2] employing gallic acid as a standard. TPC is expressed as μg gallic acid equivalents (GAE) mL^{-1}.

A randomized block design with three blocks and three treatments was used. Each experimental plot consisted of three trees bordered by two guard rows. Treatments consisted of a winter application (middle June) of three levels of raw OMWW application (80, 160 and 500 m^3 ha^{-1}) and one control treatment (not amended). From each replicate, nine 5-cm diameter soil cores from the surface (0–20 cm) were randomly collected to make a composite sample.

Soil sampling was conducted 10, 30 and 90 days after the OMWW application. After collecting the soil samples, they were immediately stored in sealed plastic bags in a cooler and transported to the laboratory. Water pH, electric conductivity (using soil water ratios of 1:2.5), total organic carbon (TOC, Walkey and Black), total nitrogen (TN, semi-micro-Kjeldahl) and extractable phosphorus (EP, Bray and Kurtz) were determined according to methodologies proposed by Black,[18] Bremner,[19] and Bray and Kurtz,[20] respectively. Organic matter content (OM) was quantified according to Vargas Gil et al.[21]

The field-moist samples were then sieved (3 mm pore size mesh) and stored in sealed plastic bags at 4°C for microbiological analysis. All microbial determinations were performed within one week of sampling.

Fluorescein diacetate (FDA) hydrolysis

General microbial activity was measured by fluorescein diacetate (FDA) hydrolysis using the procedure proposed by Adam and Duncan.[22] Briefly, 2 g of soil and 15 mL of 60 mM potassium phosphate buffer pH 7.6 were placed in a 50 mL conical flask. Substrate (FDA, 1000 μg mL^{-1}) was added to start the reaction, and the flask contents were shaken by hand. The flasks were then placed in an orbital incubator at 30°C for 20 min at 100 rpm. Once removed from the incubator, 15 mL of chloroform/methanol (2:1 v/v) was immediately added to terminate the reaction. Stoppers were replaced on the flasks and the contents were thoroughly shaken by hand. The contents of the conical flasks were then transferred to 50 mL centrifuge tubes and centrifuged at 2000 rpm for 3 min. The supernatant from each sample was then filtered into 50 mL conical flasks and the filtrates measured at 490 nm on a spectrophotometer.

Microbial community structure

The microbial community structure of the soil was determined by analyzing its phospholipid fatty acid composition using a modification of the Bossio and Scow method.[23] Each soil sample (10 g dry soil) was extracted overnight with 23 mL of one-phase buffer containing 1:2:0.8 ratio of chloroform, methanol, and phosphate buffer (8.7 g K_2HPO_4 L^{-1}, pH 7.4). The total lipid extract was fractioned into neutral lipids, glycolipids, and polar lipids by silicic acid chromatography [24–25] and the polar lipid fraction containing the phospholipids was isolated and transesterified into fatty acid methyl esters using a mild acid methanolysis reaction. Fatty acid methyl esters were analyzed by capillary gas chromatography with flame ionization detection on a PerkinElmer (Clarus 500 GC) using a 30 m non-polar column (Col-Elite-5) with both the injector and detector maintained at 290°C. The column temperature

was programmed to start a 180°C for 4 min and then ramp up at a rate of 4–280°C. Methyl nonadecanoate was used as a quantitative internal standard. The separated fatty acid methyl-esters were identified and quantified by chromatography retention time, using standard bacterial acid methyl ester mix (Supelco, Supelco UK, Poole, Dorset, UK). For each sample the abundance of individual fatty acid methyl-esters was expressed as nmol PLFA g^{-1} dry soil.

The fatty acid nomenclature used is as follows: total number of carbon atoms: number of double bonds, followed by the position (x) of the double bond from the methyl end of the molecule. Cis and trans configurations are indicated by c and t, respectively. Anteiso- and isobranching are designated by the prefix a or i. The methyl group known as 10 Me is on the 10th carbon atom from the carboxyl end of the molecule. Cy indicates cyclopropane fatty acids. Br indicates a branched fatty acid with unknown branching configuration.

Fruit and oil yield

At harvest time, each individual tree was hand-harvested and fruit production was quantified. From each tree, three independent fruit samples (500 g each) were taken in order to determine the oil yield.

Statistical analysis

Data were analyzed using a two-way ANOVA to determine the combined effects of different levels of raw OMWW and exposure times on each variable, using InfoStat software, Version 2011[26]. The significance of differences was recorded at a level of 0.05. Whenever ANOVA indicated a significant difference, a comparison of means by least significant difference (LSD) was carried out. A multivariate statistical analysis of the data set from the soil microbial community test was performed using principal component (PC) analysis. Finally, correlation analysis was done employing Pearson's analysis.

Results and discussion

OMWW physico-chemical properties and short-term effects of three OMWW dosages on soil properties and productive parameters of olive trees

The sampled OMWW pH was acid (4.83) with an intermediate electrical conductivity (11.5 dS m^{-1}). BOD_5 and COD values were moderate (65.4 y 29.3 g O_2 L^{-1}, respectively) and total phenolic content was relatively low (4.36 μg gallic acid mL^{-1}). In general terms, the values recorded for these parameters are within the ranges of those in the literature.[11–14,27–28]

Table 2 details the evolution of soil physico-chemical parameters for 90 days after OMWW application, showing that these properties changed significantly as a result of the OMWW application.

Different doses of OMWW had different effects on soil characteristics according to each examined time period. In general, treatment with the three OMWW doses (T1, T2 and T3) did not significantly modify soil pH values with respect to the control treatment. These values recorded for the studied soils reflect the fact that their chalky nature makes them neutralize the acidity of the OMWW with the

Table 2. Evolution along time of mean values of physico-chemical parameters after treatment with three dosages of OMWW.

	Day 10				*Day 30*				*Day 90*							
Parameter	*T1*	*T2*	*T3*	*CO*	*T1*	*T2*	*T3*	*CO*	*T1*	*T2*	*T3*	*CO*	*LSD*	*Treatment*	*Day*	*Treatment × Day*
pH	8.26	8.44	8.22	8.09	7.58	8.24	7.73	8.11	8.23	8.16	8.28	8.06	0.53	nsd‡	**†	nsd
Organic matter content (g 100 g^{-1})	3.67	3.62	4.23	3.20	3.93	3.31	4.68	2.40	3.31	3.03	3.47	2.60	0.94	***§	**	nsd
Extractable phosphorus (ppm)	>70	60.3	>70	23.3	59.3	61.0	>70	21.0	40.7	59.3	>70	26.0	12.9			**
Total organic carbon (g 100 g^{-1})	2.13	2.09	2.46	1.86	2.28	1.92	2.72	1.40	1.92	1.73	1.92	1.51	0.55	***	**	nsd
Kjeldahl nitrogen (g 100 g^{-1})	0.18	0.19	0.20	0.17	0.20	0.17	0.23	0.13	0.17	0.15	0.17	0.13	0.05	***	**	nsd
Electrical conductivity (dS m^{-1})	3.17	3.03	4.83	0.73	3.27	2.40	5.97	0.70	1.63	1.43	4.43	1.00	1.32	***	**	nsd
C/N ratio	11.7	11.3	12.1	10.7	11.6	11.1	12.0	11.0	11.3	11.3	11.3	11.4	1.10	***	***	nsd

Mean values (n = 3).
‡ nsd, indicates no significant difference. † Significant at $P \leq 0.05$. § Significant at $P \leq 0.01$.

Table 3. Fruit and oil yields from cv. Manzanilla (*Olea europaea* L.) grown under different OMWW irrigation levels.

	Treatments			
Parameters	*T1*	*T2*	*T3*	*CO*
Fruit yield (Kg tree^{-1})	$75.6^{a} \pm 8.56$	$72.3^{a} \pm 9.12$	$76.4^{a} \pm 7.52$	$78.1^{a} \pm 8.77$
Oil yield (Kg tree^{-1})	$11.5^{a} \pm 1.31$	$11.0^{a} \pm 1.39$	$11.4^{a} \pm 1.12$	$11.5^{a} \pm 1.52$

Mean values (n = 3) ± standard deviation values.
Values in each row with same superscript letters not present significant differences ($P \leq 0.05$) among treatments.

alkalinity of their carbonates. Although there are several precedents which justify that OMWW infiltration causes carbonate solution, thus changing the pH,[11,29–32] in this work OMWW's effect on pH was insignificant. This can be attributed to the large amount of carbonates present as a fine powder throughout the soil profile, particularly in the deeper original material (LOESS). When applying different doses of OMWW, Aguilar [33] and Mechri et al.[12] found similar results in the calcareous soils of southern Spain and Tunisia.

As evidenced shortly after the treatment, OM increased significantly and proportionally to the strength of the OMWW dose applied. TOC also experienced a statistically marked variation based on the dosage used, with a high correlation between the two parameters (r = 0.98, $P = 0{,}001$).

The time evolution of assimilable phosphorus concentration in the soil exhibited a behavior similar to that of OM content, with a significant correlation in the two parameters (r = 0.68, $P < 0.0001$). Extractable phosphorus and total nitrogen contents also increased with the applied dosage. Both tended to decrease with time; these values were considerably higher on day 10 than on day 90 (Table 2).

The high amount of organic carbon in OMWW altered the C/N ratio, so the irrigation with this waste immobilizes inorganic nitrogen. Moreover, the presence of phenol compounds in OMWW could also inhibit the N mineralization and consequently the different forms of its availability.[33]

As pointed out by other authors,[11–14] the C/N ratio increased with larger doses of OMWW. However, 90 days into the experiment, there was no difference between the different treatments, most likely because of the decrease of TOC in the soil.

The concentration of assimilable phosphorus in the soil can be attributed to OMWW reductive conditions that change Fe $^{+}3$ into soluble ferrous phosphates and increase the available phosphorus or to the mineralization of phosphorus organic forms in OMWW or to the extraction of the plant. However, the amount of phosphorous that may be washed is small since the treatment was carried out during a time of limited rainfall (Fig. 1).

These increments are consistent with the results of those assays where byproducts derived from two [34–37] or three-phase oil mills [38] are used. Hence, the direct application of OMWW, even at low doses, could be a feasible alternative to phosphorus commercial fertilizers. López-Piñeiro et al.[36] also suggested that this phosphorus increment might improve olive tree tolerance to the water stress of the dry season. However, other authors [39] state that OMWW treatment would not build up the amount of phosphorus and nitrogen in the plant, perhaps because of the strong shock it would suffer when its roots come into contact with the OMWW.

High dose application of OMWW had a negative effect on soil salinity. Electrical conductivity in the soils treated with the highest concentration surpassed the established limit of 4 dSm^{-1} for saline soils.

The increase of electric conductivity provoked by these doses has already been observed when OMWW or solid parts of the olive or "alperujo" were used.[11,14,32,40] Even though olive trees tolerate salinity, it must be taken into account that the application was carried out near the winter season and in a kind of climate when salt is not washed away. Thus, plant susceptibility to cold might increase as a result of the phytotoxic effect of the salts present.[41] Hence, high doses of OMWW are harmful for the plants.

Fruit and oil yield

Despite the previously mentioned findings, effects on productive parameters were not observed (Table 3). This is probably because the time between the OMWW

Table 4. Microbial activity (μg fluorescein g^{-1} soil) in soil treated with different dosages of olive mill wastewater (OMWW) at 10, 30 and 90 days after application.

	Days of incubation		
Treatments with OMWW	*Day 10*	*Day 30*	*Day 90*
Control	$0.62^{a} \pm 0.04$	$1.29^{a} \pm 0.17$	$0.80^{a} \pm 0.07$
T1: (80 m^3 ha^{-1})	$2.10^{b} \pm 0.12$	$1.72^{ab} \pm 0.19$	$1.98^{b} \pm 0.32$
T2: (160 m^3 ha^{-1})	$2.20^{b} \pm 0.14$	$1.63^{ab} \pm 0.07$	$1.55^{ab} \pm 0.09$
T3 (500 m^3 ha^{-1})	$2.30^{b} \pm 0.10$	$1.98^{b} \pm 0.12$	$2.88^{c} \pm 0.36$

Mean values (n = 3) ± standard deviation values.
Values in each row with different superscript letters present significant differences ($P \leq 0.05$) among days of incubation.

Table 5. Soil microbial community as estimated by PLFA analysis after treatment with three dosages of OMWW.

	Day 10				*Day 30*				*Day 90*							
	T1	*T2*	*T3*	*CO*	*T1*	*T2*	*T3*	*CO*	*T1*	*T2*	*T3*	*CO*	*LSD*	*Treatment*	*Day*	*Treatment × Day*
Total PLFAs	114.9	151.5	133.1	111.4	124.7	138.8	154.7	119.4	130.5	140.9	155.8	139.9	6.6			***
Gram (+)	11.0	11.1	3.43	12.9	11.4	8.2	1.2	14.2	11.4	11.7	8.17	13.1	0.7			***
Gram (−)	30.9	34.7	49.1	32.2	33.1	40.3	50.3	36.1	34.6	41.8	50.1	37.6	3.5	***	***	nsd
Fungi	10.4	13.4	14.9	10.9	11.5	10.8	13.1	14.5	10.9	12.2	10.1	11.0	2.1			***
Structural group																
Saturated	60.1	90.4	64.7	53.3	67.3	78.0	88.0	53.0	72.3	73.5	85.5	76.4	6.3			***
Unsaturated	11.0	11.1	3.4	12.9	11.4	8.2	1.2	14.2	11.4	11.7	8.17	13.1	0.7			***
Hydroxylated	2.4	1.8	0.9	1.8	1.3	1.3	2.0	1.5	1.2	1.6	1.9	1.7	0.5			***
Monounsaturated.	21.9	25.1	35.8	22.7	24.1	29.27	37.0	27.5	24.3	30.6	37.7	28.2	3.5	***	***	nsd
Polyunsaturated	10.4	13.4	14.9	10.9	11.5	10.8	13.1	14.5	10.9	12.2	10.1	11.0	2.1			***
Cyclopropyl group	9.0	9.6	13.2	9.5	8.9	11.1	13.3	8.5	10.3	11.2	12.3	9.4	0.6			***
Class ratios																
Saturat./Unsaturat.	1.9	2.4	1.3	1.6	1.9	2.0	1.8	1.2	2.1	1.7	1.8	2.0	0.3			***
Gram(+)/Gram(−)	0.4	0.3	0.1	0.4	0.3	0.2	0.0	0.4	0.3	0.3	0.2	0.4	0.1			***
Bacteria/fungi ratio	4.0	3.5	3.5	4.1	3.9	4.5	3.9	3.4	4.2	4.4	5.7	4.6	0.7			**

Mean values (n = 3).
‡nsd, indicates no significant difference. †Significant at $P \leq 0.05$. §Significant at $P \leq 0.01$.

application and the productive phase (flowering and fruit set) was longer than 5 months. This fact, coupled with the washing effect caused by the first spring rains, contributed to diminish the effect of OMWW application (Fig. 1).

Microbial activity

The OMWW treatment altered soil microbial activity (Table 4). On day 10 of incubation, the untreated soil (T0; control) showed the least microbial activity. On day 30 there

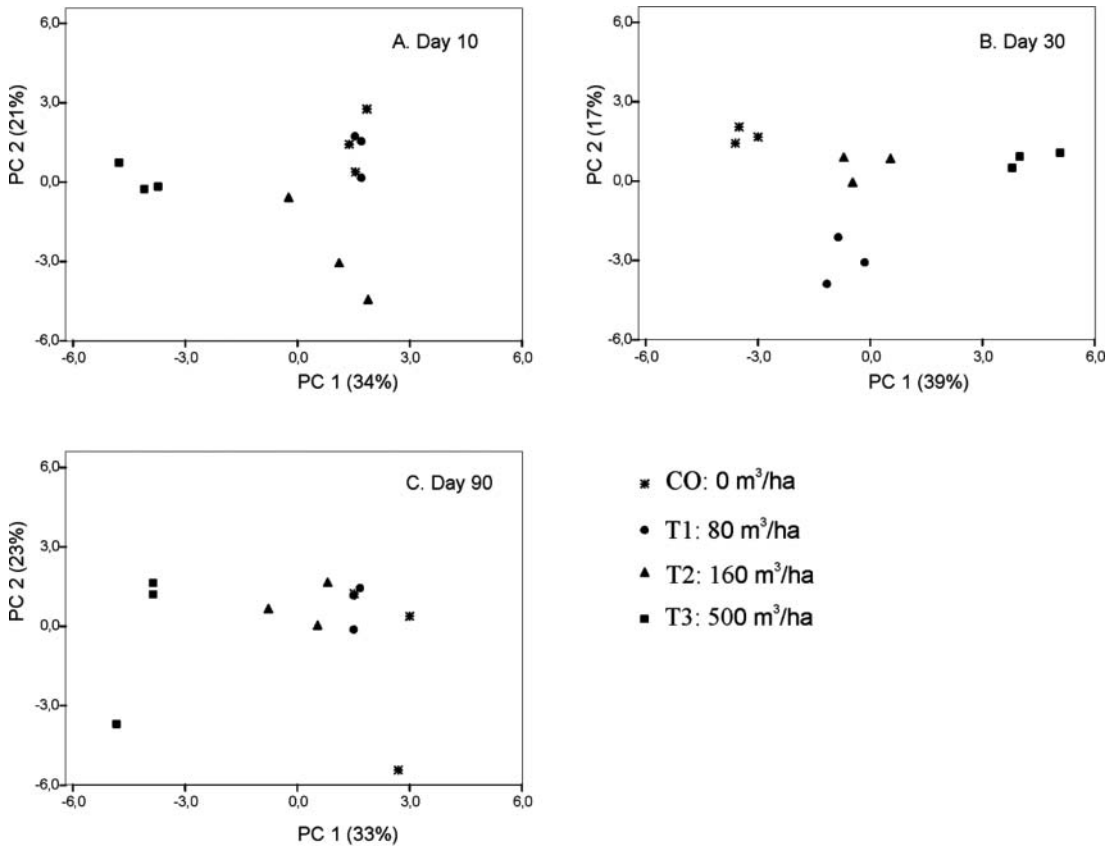

Fig. 2. Principal component analysis of soil microbial community as estimated by PLFA profiles after 10 (A), 30 (B), and 90 (C) days after treatment with three dosages of OMWW.

Table 6. Community PLFAs significantly correlated ($P \leq 0.05$) with principal components 1 and 2 of PC plots.

	PC1		*PC2*	
Day after treatment	*PLFA*	*Correlation value*	*PLFA*	*Correlation value*
Day 10	cy19:0	−0.96	16:0	−0.85
	cy17:0	−0.94	18:0	−0.85
	18:1ω7c	−0.92	12:0 3OH	−0.69
	18:2ω6,9	−0.80	17:0	−0.62
	i16:0	0.64	11:0	0.63
	12:0	0.83		
	a15:0	0.87		
	14:0 3OH	0.93		
	i15:0	0.97		
Day 30	a15:0	−0.96	i17:0	0.63
	i15:0	−0.93	18:1ω7c	0.67
	i16:0	−0.93	11:0	0.68
	12:0	−0.77	14:0	0.92
	14:0 3OH	0.61		
	18:1ω7c	0.68		
	16:1ω9c	0.72		
	cy17:0	0.85		
	cy19:0	0.88		
	18:0	0.91		
	16:0	0.92		
Day 90	cy19:0	−0.90	10:0 2OH	−0.91
	cy17:0	−0.83	12:0 2OH	−0.87
	18:1ω7c	−0.82	17:0	−0.79
	16:1ω9c	−0.80	i17:0	0.79
	18:0	−0.75	14:0	0.68
	16:0	−0.64	13:0	0.67
	i15:0	0.68		
	a15:0	0.85		
	i16:0	0.95		

was a similar trend, although not as substantial. Intermediate OMWW concentrations (T1, 80 m^3 ha^{-1}; T2, 160 m^3 ha^{-1}) did not differ significantly from control. Towards day 90, the soil with the highest OMWW concentration (T3, 500 m^3 ha^{-1}) showed the highest microbial activity.

Fluorescein diacetate assay (FDA) hydrolysis is widely used as an indicator of total microbial activity because the technique includes the participation of non-specific enzymes like esterase, protease and lipase.[22] Other authors have suggested that there is a close correlation between the evaluation of FDA hydrolysis and the total microbial biomass.[42] Enzyme activity in the soil is important, as it can indicate the soil's potential to facilitate basic biochemical processes and nutrient cycle.[43] Piotrowska et al.[13] observed a slight variation in FDA shortly after applying OMWW. In the present work microbial activity as hydrolyzed FDA per gram of soil increased significantly after a treatment of OMWW. The soil samples that had previously been treated with OMWW had a high microbial activity from the beginning until the end of the incubation period. These results suggest that the application of the available carbon present within the OMWW promotes the growth of indigenous microorganisms and/or results in an increase in the synthesis of non-specific enzymes. Similar results that indicate an increase in hydrogenase activity shortly after OMWW treatment have been reported by other authors.[13]

Microbial community structure

Although OMWW can improve soil quality and the amount of heavy metals and pathogenic organisms it contains is negligible, there are some disadvantages in connection with acidity, salinity, lipids, organic acids and phenolic compounds.[44] The abundant presence of high molecular weight polyphenols after a Mn or Fe-mediated oxidation may inhibit fungi and/or bacteria. Additionally, OMWW decomposition by soil microorganisms can induce an oxygen decrease on the soil surface and inhibit aerobic microbial activity.[44] Our results show that OMWW application modified the microbial community structure of the soil, especially 30 days after incubation (Table 5). OMWW treatment in high concentrations brought about a significant decrease in ramified fatty acids that are widely used as Gram positive bacteria indicators. The soil treated with this same OMWW concentration underwent an increase in Gram negative bacteria.

The principal component (PC) analysis showed a variance of 55, 56 and 56% for days 10, 30 and 90 respectively (Fig. 2). This analysis revealed a clear difference of treatment T3 when compared with the others. This difference was much more evident on day 30 of incubation. On this particular day, separation between the treatments was the most marked out of all the OMWW concentrations assayed. The sample with the highest OMWW concentration (T3; 500 m^3 ha^{-1}) was associated with an increase of some cyclopropanoic and monoinsaturated fatty acids (Tables 5 and 6). On the contrary, untreated soil (T0, control) showed high levels of ramified fatty acids that are widely used as Gram positive bacteria indicators (Tables 5 and 6).

These results are consistent with the findings made by other authors,[12,45] who reported a growth in Gram negative bacteria and a reduction of Gram positive bacteria 30 days after an application of OMWW 150 m^3 ha^{-1}. Others have reported similar results.[46] Even more controversial was the effect of OMWW on fungal biomass (Table 5). Bonanomi et al.[47] carried out *in vitro* studies on the effect of dry olive residues on fungi growing on Petri dishes and concluded that these remnants increased the radial growth of some phytopathogenic fungi. These authors found that olive residues can provide energy and nutrients for the growth of saprophytic fungi. However, Obied et al.[46] reported that the studied fungi were not affected by any of the extracts assayed. In our study, the OMWW application did not cause consistent changes in the fungal biomass during the analyzed time frame. It is

a well-known fact that antifungal activity requires considerable lipophilicity. OMWW is essentially hydrophilic, as the more lipophilic constituents remain in the olive oil. Finally, OMWW impact on the microbiota may cause a temporary soil enrichment with the addition of easily available C, or it may bring about the inhibition of some important microorganisms for nutrient cycling.[11,48] Our study suggests that OMWW significantly increased the total microbiota. This OMWW effect on the microbial biomass could be the result of an increment in soil fertility, considering that the enriching properties of residual waters, with their high content of organic matter, P, N, and K. OMWW might also have been used as a source of bioactive components.

Conclusions

Agricultural treatment with OMWW proportionally increases the organic matter content, organic carbon, total nitrogen and extractable phosphorus in the soil. Amendment with this byproduct can further promote soil activity and microbial biomass. However, an OMWW application increases the soil salinity before the dry and cold season which is characteristic of the studied area. In spite of this, productive parameters were not significantly affected. We suggest that a low dose OMWW treatment in olive groves can be an economical and simple practice without marked unfavorable side effects.

Finally, further studies are necessary to analyze the long-range sustainability of OMWW treatment in olive groves and to examine questions concerning specific plant physiology and soil biology with greater detail.

Acknowledgments

This work was financed with grants from Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Secretaría de Ciencia y Tecnología de la Universidad Nacional de Córdoba (SeCyT-UNC), Instituto Nacional de Tecnología Agropecuaria (INTA), and Ministerio de Ciencia y Tecnología de la provincial de Córdoba (Argentina). Pierluigi Pierantozzi is a fellow, Mariela Torres, José Meriles and Damián Maestri are career researchers at CONICET (Argentina), Mariela Torres and María Basanta are professionals from INTA (Argentina).

References

[1] Gomez del Campo, M.; Morales-Sillero, A.; Vita Serman, F.; Rousseaux, M.C.; Searles, P. El Olivar en los valles áridos del Noroeste de Argentina (provincia de Catamarca, La Rioja y San Juan). Olivae **2010**, *114*, 23–45.

[2] Torres, M.M.; Pierantozzi, P.; Cáceres, M.E.; Labombarda, P.; Fontanazza, G.; Maestri, D.M. Genetic and chemical assessment of Arbequina olive cultivar grown in Córdoba province, Argentina. Journal of the Science of Food and Agriculture **2009**, *89*, 523–530.

[3] Aktas, E.S.; Imre, S.; Ersoy, L. Characterization and lime treatment of olive mill wastewater. Water Research **2001**, *35*, 2336–2340.

[4] Sierra, J.; Martí, E.; Montserrat, G.; Cruaas, R.; Garau, M.A. Characterisation and evolution of a soil affected by olive oil mill wastewater disposal. Science of the Total Environment **2001**, *279*, 207–214.

[5] Komilis, D.P.; Karatzas, E.; Halvadakis, C.P. The effect of olive mill wastewater on seed germination after various pretreatment techniques. Journal of Environmental Management **2005**, *74*, 339–348.

[6] Ben Sassi, A.; Boularbah, A.; Jaouad, A.; Walker, G.; Boussaid, A. A comparison of Olive Oil Mill Wastewaters (OMW) from three different processes in Morocco. Process Biochemistry **2006**, *41*, 74–78.

[7] Roig, A.; Cayuela, M.L.; Sánchez-Monedero, M.A. An overview on olive mill wastes and their valorisation methods. Waste Management **2006**, *26*, 960–969.

[8] El Hajjouji, H.; Pinelli, E.; Guiresse, M.; Merlina, G.; Revel, J.C.; Hafidi, M. Assessment of the genotoxicity of olive mill waste water (OMWW) with the Vicia faba micronucleus test. Mutation Research-Genetic Toxicology and Environmental Mutagenesis **2007**, *634*, 25–31.

[9] Parinos, C.S.; Stalikas, C.D.; Giannopoulos, T.S.; Pilidis, G.A. Chemical and physicochemical profile of wastewaters produced from the different stages of Spanish-style green olives processing. Journal of Hazardous Materials **2007**, *145*, 339–343.

[10] Pierantozzi, P.; Zampini, C.; Torres, M.; Isla, M.I.; Verdenelli, R.A.; Meriles, J.M.; Maestri, D. Physico-chemical and toxicological assessment of liquid wastes from olive processing-related industries. Journal of the Science of Food and Agriculture **2012**, *92*, 216–223.

[11] Di Serio, M.G.; Lanza, B.; Mucciarella, M.R.; Russi, F.; Iannucci, E.; Marfisi, P.; Madeo, A. Effects of olive mill wastewater spreading on the physico-chemical and microbiological characteristics of soil. International Biodeterioration and Biodegradation **2008**, *62*, 403–407.

[12] Mechri, B.; Echbili, A.; Issaoui, M.; Braham, M.; Elhadj, S.B.; Hammami, M. Short-term effects in soil microbial community following agronomic application of olive mill wastewaters in a field of olive trees. Applied Soil Ecology **2007**, *36*, 216–223.

[13] Piotrowska, A.; Iamarino, G.; Rao, M.A.; Gianfreda, L. Short-term effects of olive mill waste water (OMW) on chemical and biochemical properties of a semiarid Mediterranean soil. Soil Biology and Biochemistry **2006**, *38*, 600–610.

[14] Piotrowska, A.; Rao, M.A.; Scotti, R.; Gianfreda, L. Changes in soil chemical and biochemical properties following amendment with crude and dephenolized olive mill waste water (OMW). Geoderma **2011**, *161*, 8–17.

[15] Cabrera, A.L. Regiones fitogeográficas argentinas. In *Enciclopedia argentina de agricultura y jardineria*; Kugler, W.F., Ed.; Argentina, 1994; 2, 1–85.

[16] AOCS. In *Official methods and recommended practices of the American Oil Chemists' Society*; Firestone, D., Ed.; Author: Urbana, IL, 2009; 6, 1–1200.

[17] Macheix, J.J.; Fleuriet, A.; Billot, J. In *Fruit Phenolics*; CRC Press: Boca Raton, FL, 1990; 1–378.

[18] Black, C.A. In *Methods of Soil Analysis*; Black, C.A., Ed., Madison, WI, 1965; 2, 985–996.

[19] Bremner, J.M. In *Methods of soil analysis*; Sparks, D.L., Ed.; Madison, WI, 1996; 3, 1085–1121.

[20] Bray, R.H.; Kurtz, L.T. Determination of total, organic and available forms of phosphorus in soil. Soil Science **1945**, *59*, 39–45.

[21] Vargas Gil, S.; Meriles, J.; Conforto, C.; Figoni, G.; Basanta, M.; Lovera, E.; March, G.J. Field assessment of soil biological and chemical quality in response to crop management practices. World Journal of Microbiology and Biotechnology **2009**, *25*, 439–448.

[22] Adam, G.; Duncan, H. Development of a sensitive and rapid method for the measurement of total microbial activity using fluorescein diacetate (FDA) in a range of soils. Soil Biology and Biochemistry **2001**, *33*, 943–951.

[23] Bossio, D.A.; Scow, K.M. Impacts of carbon and flooding on soil microbial communities: Phospholipid fatty acid profiles and substrate utilization patterns. Microbial Ecology **1998**, *35*, 265–278.
[24] Guckert, J.B.; Antworth, C.P.; Nichols, P.D.; White, D.C. Phospholipid, ester-linked fatty acid profiles as reproducible assays for changes in prokaryotic community structure of estuarine sediments. FEMS Microbiology Letters **1985**, *31*, 147–158.
[25] McKinley, V.L.; Peacock, A.D.; White, D.C. Microbial community PLFA and PHB responses to ecosystem restoration in tallgrass prairie soils. Soil Biology and Biochemistry **2005**, *37*, 1946–1958.
[26] Di Rienzo, J.A., Casanoves, F., Balzarini, M.G., Gonzalez, L., Tablada, M. and Robledo, C.W. *InfoStat versión 2011*. Grupo InfoStat, F., Universidad Nacional de Córdoba: Córdoba, Argentina; 2011. Available at http://www.infostat.com.ar (accessed June 2012).
[27] Justino, C.I.; Duarte, K.; Loureiro, F.; Pereira, R.; Antunes, S.C.; Marques, S.M.; Gonçalves, F.; Rocha-Santos, T.A.P.; Freitas, A.C. Toxicity and organic content characterization of olive oil mill wastewater undergoing a sequential treatment with fungi and photo-Fenton oxidation. J. Hazard. Mat. **2009**, *172*, 1560–1572.
[28] Mechri, B.; Issaoui, M.; Echbili, A.; Chehab, H.; Mariem, F.B.; Braham, M.; Hammami, M. Olive orchard amended with olive mill wastewater: Effects on olive fruit and olive oil quality. J. Hazard. Mat. **2009**, *172*, 1544–1550.
[29] Marsilio, V.; DiGiovacchino, L.; Solinas, M.; Lombardo, N.; Bati, B. First observations on the disposal effects of olive mills vegetation waters on cultivated soil. Acta Hort. **1990**, *286*, 493–496.
[30] Proietti, P.; Palliotti, A.; Tombesi, A. and Cenci, G. Chemical and microbiological modifications of two different cultivated soils by olive oil waste water administration. Agricoltura Mediterranea **1995**, *125*, 160–171.
[31] Di Giovacchino, L.; Marsilio, V.; Costantini, N.; Di Serio, M.G. In *Use of olive mill wastewater (OMW) as fertilizer of the agricultural soil: effects on crop production and soil characteristics*, Proceedings of the Third European bioremediation conference, Chania, Crete, Greece, July 4–7, 2005, 165–169.
[32] Kavdir, Y.; Killi, D. Influence of olive oil solid waste applications on soil pH, electrical conductivity, soil nitrogen transformations, carbon content and aggregate stability. Bioresource Technology **2008**, *99*, 2326–2332.
[33] Aguilar, M.J. Olive oil mill wastewater for soil nitrogen and carbon conservation. J. Environmental Management **2009**, *90*, 2845–2848.
[34] Montemurro, F.; Convertini, G.; Ferri, D. Mill wastewater and olive pomace compost as amendments for rye-grass. Agronomie **2004**, *24*, 481–486.
[35] Madejón, E.; Burgos, P.; López, R.; Cabrera, F. Agricultural use of three organic residues: Effect on orange production and on properties of a soil of the 'Comarca Costa de Huelva' (SW Spain). Nutrient Cycling in Agroecosystems **2003**, *65*, 281–288.
[36] López-Piñeiro, A.; Albarrán, A.; Cabrera, D.; Peña, D.; Nunes, J.M.R. De-oiled two-phase olive mill waste application impact on phosphorus sorption and fractionation in a mediterranean soil under olive production. Soil Sci. **2011**, *176*, 22–32.
[37] López-Piñeiro, A.; Albarrán, A.; Nunes, J.M.R.; Barreto, C. Short and medium-term effects of two-phase olive mill waste application on olive grove production and soil properties under semiarid mediterranean conditions. Bioresource Tech. **2008**, *99*, 7982–7987.
[38] Tejada, M.; Gonzalez, J.L. Effects of application of a by-product of the two-step olive oil mill process on maize yield. Agronomy J. **2004**, *96*, 692–699.
[39] Mechri, B.; Cheheb, H.; Boussadia, O.; Attia, F.; Ben Mariem, F.; Braham, M.; Hammami, M. Effects of agronomic application of olive mill wastewater in a field of olive trees on carbohydrate profiles, chlorophyll a fluorescence and mineral nutrient content. Environmental and Experimental Botany **2011**, *71*, 184–191.
[40] Paredes, M.J.; Moreno, E.; Ramos-Cormenzana, A.; Martinez, J. Characteristics of soil after pollution with waste waters from olive oil extraction plants. Chemosphere **1987**, *16*, 1557–1564.
[41] Turchetti Iturrieta, J.P. Efecto del estrés hídrico y salino sobre la tolerancia al frío en plantas jóvenes de olivo (Olea europaea L.) cv. Arbequina y Barnea. Instituto Nacional de Tecnología Agropecuaria, Universidad Nacional de San Juan-Facultad de Ingeniería, Departamento Agronomía, Tesis de Grado; 2011; 1–68.
[42] Sánchez-Monedero, M.A.; Mondini, C.; Cayuela, M.L.; Roig, A.; Contin, M.; De Nobili, M. Fluorescein diacetate hydrolysis, respiration and microbial biomass in freshly amended soils. Biology and Fertility of Soils **2008**, *44*, 885–890.
[43] Pascual, I.; Antolín, M.C.; García, C.; Polo, A.; Sánchez-Díaz, M. Effect of water deficit on microbial characteristics in soil amended with sewage sludge or inorganic fertilizer under laboratory conditions. Bioresource Technology **2007**, *98*, 29–37.
[44] Sierra, J.; Martí, E.; Garau, M.A.; Cruañas, R. Effects of the agronomic use of olive oil mill wastewater: Field experiment. Science of the Total Environment **2007**, *378*, 90–94.
[45] Mechri, B.; Mariem, F.B.; Baham, M.; Elhadj, S.B.;Hammami, M. Change in soil properties and the soil microbial community following land spreading of olive mill wastewater affects olive trees key physiological parameters and the abundance of arbuscular mycorrhizal fungi. Soil Biology and Biochemistry **2008**, *40*, 152–161.
[46] Obied, H.K.; Bedgood, Jr., D.R.; Prenzler, P.D.; Robards, K. Bioscreening of Australian olive mill waste extracts: Biophenol content, antioxidant, antimicrobial and molluscicidal activities. Food and Chemical Toxicology **2007**, *45*, 1238–1248.
[47] Bonanomi, G.; Giorgi, V.; Giovanni, D.S.; Neri, D.; Scala, F. Olive mill residues affect saprophytic growth and disease incidence of foliar and soilborne plant fungal pathogens. Agriculture, Ecosystems and Environment **2006**, *115*, 194–200.
[48] Saadi, I.; Laor, Y.; Raviv, M.; Medina, S. Land spreading of olive mill wastewater: Effects on soil microbial activity and potential phytotoxicity. Chemosphere **2007**, *66*, 75–83.