J. Agr. Sci. Tech. (2016) Vol. 18: 1883-1896

Olive Mill By-products Application: Organic Olive Orchard Yield Performance and Soil Fertility

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ABSTRACT

Seasonal accumulation and incorrect disposal of olive mill by-products may be detrimental to the environment. Conversely, their proper recycling as soil amendments may be a sustainable solution. Therefore, the objectives of this three-year field trial were: (i) Investigating the effects of Olive Mill Wastewater (OMW) and Pomace Compost (PC) on plant nutritional status and yield in an organic olive grove; (ii) The impact on main soil properties, and (iii) Verifying if these experimental fertilizers can replace the widespread fertilization practices. The OMW and PC treatments were compared to a commercial Organic-mineral Fertilizer (OF) and green manure of horse bean (MV). Plant nutritional status, soil properties and agronomical performance of treatments were assessed. The OMW and OF determined yield that was on average significantly higher than MV and PC by 191 and 55%, respectively. The best leaves P contents in PC indicated a more effective release of this nutrient as compared to the other treatments, which can be matched with more favorable soil conditions. The comparable yield and leaves composition for OMW and OF suggested the possibility to replace the OF with the OMW. The effects on olive oil production and soil fertility highlighted that OMW (and PC as a second choice) could be applied to sustain olive tree production, substituting traditional fertilizers.

Keywords: Composting, Olive grove soils, Olive mill wastewater, Olive pomace**,** Organic farming.

[DOR: 20.1001.1.16807073.2016.18.7.4.4]

INTRODUCTION

In the Mediterranean countries, the olive (*Olea europaea* L.) oil agro-industry generates large amounts of different wastes. Olive pomace and Olive Mill Wastewater (OMW) are the by-products of the three-phase continuous centrifugation (Saadi *et al.,* 2007). By contrast, the modern two-phase system produces a lignocellulosic olive pomace, which is a humid, solid by-product with high contents of water (56.6-74.5%), phenols and lipids (Chowdhury *et al.,* 2013). The seasonal accumulation of these mill by-products, as well as their incorrect disposal, may represent potential threats to the environment (Niaounakis and Halvadakis, 2006; Kapellakis *et al.,* 2008). In fact, the two-phase olive pomace is often rich in potentially phytotoxic and bacteriostatic substances (e.g. polyphenols and organic acids) and elevated salt concentrations that may cause negative impacts on crops and environment, when the pomace is applied to the soil (Gigliotti *et al.,* 2012). However, pomace also contains a large amount of organic matter and nutrients that could be recycled in agriculture, by mixing and composting this by-product with bulking materials (e.g., cereal straw or

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pruning wastes) (Montemurro *et al.,* 2009). This last aerobic degradation process could drastically reduce the potential phytotoxic compounds of the olive mill by-products (Diacono *et al.,* 2012).

The proper direct application of OMW to the soil, as an organic fertilizer, has been proposed as a low cost method to recycle it (Montemurro *et al.,* 2004). The Italian Law (n. 574, 1996) allows soil spreading of OMW for agronomical use with maximum dosage of 80 m^3 ha^{-1} yr^{-1} (for continuous centrifuge system). The beneficial effects of OMW on the yield of some crops indicated that it may become a very important source of nutrients and organic matter in agriculture (Barbera *et al.,* 2013). In particular, according to Cayuela *et al.* (2010), in the southern regions of the Mediterranean area the soil organic matter content is extremely low. This condition is highly correlated with soil fertility reduction, due to negative effects on physical, chemical and biological properties of soil. Therefore, properly recycling the organic agro-industrial wastes as soil amendments may represent a solution for their disposal, to replace the losses of organic matter (López-Piñeiro *et al.,* 2011). However, to this date the effects of olive mill by-products on olive orchards have not been exhaustively studied, particularly in organic olive-growing systems. Using olive mill by-products in these systems would create a virtuous cycle of biomasses return to the soil within the same production chain. Moreover, several olive grove soils have low organic matter content, thus it could be interesting to define the replacement of the most commonly used methods of fertilization by the organic fertilizers from the agro-food wastes. Based on these considerations, the objectives of this three-year field trial in an organic Mediterranean olive grove were: (i) to investigate the effects of alternative soil organic amendments/fertilizers, particularly OMW and olive Pomace Compost (PC), on plant nutritional status and yield; (ii) To assess the impact of the tested experimental treatments on main soil properties after repeated applications, and (iii) To verify if the use of olive mill wastes can be sustainable within actual cultivation systems, substituting the practice of soil fertility management commonly used in local olive orchards of the study area.

MATERIALS AND METHODS

Site of Field Trial

The research was carried out from 2006 to 2008 in an organic olive-growing farm at Matera (Basilicata Region, southern Italy), 250 m asl (40° 36' 42.839" N, 16° 37' 54.552" E) on a soil with 1.23 g kg^{-1} of N, 9.87 and 834 mg kg^{-1} of available P and K, respectively, and with 19.6 g kg^{-1} of organic matter. The investigated cultivars were *Dipopp* and *Ghiannara*, with planting space of 10×10 m, which is typical of the secular olive-groves of the site. The olive grove was planted in the early '900, re-grafted at the end of 1960 seconds and it has never been irrigated. Before starting the trial, the field has been uniformly managed with organic farming practices. In particular, as for fertilizer strategy, in the previous 4 years green manure of horse bean was used.

The climate is "accentuated thermomediterranean" (UNESCO-FAO classification), with winter temperatures that can fall below 0°C, summer temperatures that can rise above 40°C, and rainfall unevenly distributed during the year, being concentrated mainly in the winter months. According to the data recorded in the nearby weather station, the total rainfall values can be ranked as follows: 2008< 2007< 2006 (i.e., 423< 552< 587 mm). The highest average maximum temperature during the critical June-September period was recorded in 2008 (31.4°C) and the lowest in 2006 (30.4°C), whereas the 2006 had the highest rainfall (279 mm) and the 2007 the lowest one (212 mm). Finally, the mean temperature values during March-November period can be ranked as follows: 2007< 2006< 2008 (i.e., 24.2< 24.6< 25.0°C).

Experimental Treatments and Measurements

In a randomized block experimental design with three replications and elementary plots of 400 m^2, the following experimental treatments were compared: (i) OMW, distributed at 80 m^3 ha^{-1}; (ii) On-farm PC distributed at 60 kg $plant^{-1}$, which were compared to: (a) A commercial NPK organic-mineral fertilizer (OF; Progress micro ILSA SpA), allowed in organic agriculture, distributed at 15 kg $plant^{-1}$, and (b) Green manure of horse bean, *Vicia faba* L. *minor* Beck (MV), sown at rate of 150 kg seeds ha^{-1}. The applied OMW rate was the maximum amount allowed by the Italian law for it. The MV is one of the most used sustainable methods to supply N to olive groves in organic farming, thus it would be considered as a control, and it was applied at a rate commonly used in the investigated area. Both the PC and the OF rates were based on the N removal by the average olive tree productions over the years (Therios, 2009). All amendments (except MV) were applied annually in mid-November on the soil after the harvest, followed by surface tillage. The horse bean was sown in the first decade of November, as it is usually done in our Mediterranean conditions. It was then chopped and incorporated into the soil (about 20 cm depth) by plowing, when plants produced the first pods at the end of flowering (i.e., at the beginning of May).

In regards to the PC treatment, three on-farm composts were produced from 2006 to 2008, by mixing olive pomace (at 90.6% rate) from two phases continuous extraction system with wheat straw, chicken manure and urea, in the following rates (on fresh weight basis): 3.6, 5.3 and 0.5%, respectively. In this research, the disposal of high doses of pomace was preferred, although all the composting matrices were used in proportions so to obtain an optimal C/N ratio (about 30). In each year, the mixtures were submitted to composting in trapezoidal piles (1.5 m high with a base of 2–3 m), for about 90 days. The bio-oxidative phase was considered finished when the temperature was stable, close to the external value, and reheating did not occur. During composting, both moisture content and internal temperature of windrows were constantly monitored. To ensure biomass oxygenation, the windrows were mechanically stirred by using a tractor with front loader when the internal temperature, measured by thermocouple probe (CHEMIE, Bari, Italy) with a digital thermometer (Delta Ohm HD 9215), reached or exceeded 60°C. The moisture content in the windrow was determined by gravimetrical method, and it was maintained over 40%. To evaluate the chemical and physical characteristics of the material, four samples of about 0.5 kg each were randomly collected from different horizons of the heap. Main characteristics of the composts produced were evaluated on samples by using the official analytical methodologies reported by Pagliai and Sequi (1997) and Violante and Sequi (2000). Since we did not find differences among composts throughout the years, the mean of the compost properties (mean ± standard deviation) are reported in Table 1.

Plant nutritional status was assessed by analyzing a composite sample of the youngest mature leaves of four (one-year-old) shoots per plant, one for each cardinal point. Plant samplings were carried out each year during the following phenological stages: (i) Onset of vegetative growth; (ii) Flowering and fruit setting; (iii) Drupe formation, and (iv) Pit-hardening. On samples of ten fresh leaves, nitrate (NO_3^-) concentration of stem juice was estimated by using the rapid paper test strips of the Nitrachek reflectometer (Merck Chemicals®, Germany). Moreover, SPAD readings (a rapid and non-destructive estimate of leaf greenness) were determined by a hand-held chlorophyll meter (SPAD 502; MINOLTA), by performing three determinations in the middle of each leaf. On the ten leaves, oven dried and reduced to a fine powder, the Phosphorus (P), potassium (K), Calcium (Ca) and Magnesium (Mg) contents were

[DOR: 20.1001.1.16807073.2016.18.7.4.4]

also determined, by Inductively Coupled Plasma-Optical Emission spectrometry (ICP-OES), according to the method of Page *et al.* (1982). Since we did not find significant differences among sampling times, the average for NO_3^- and SPAD are reported in Table 2. Moreover, the agronomical performance of treatments was evaluated at harvest (in early November of 2006 and in October for the other years) by comparing the differences in yield (kg $tree^{-1}$) and olive oil production on 100 m^{-2}. Drupe, pulp and pit weights (g), pulp: pit ratio, drupe length (mm) and drupe width (mm) were further determined on a drupes sample of 200 g. In the common agricultural practice, the two olive cultivars tested are mixed to give a typical oil that is locally highly valued. As a consequence, here yield is given as mean values per tree, as well as for both leaf nutrients analysis and soil samplings a composite sample of the two cultivars (in the same rate for each treatment) was done.

Soil samplings were carried out at 0-30 cm depth before the application of fertilizers

Table 1. Main physical and chemical characteristics of olive Pomace Compost (PC) (on dry weight basis) and Olive Mill Wastewater (OMW) (mean±standard deviation).

	Moisture (%)	pH	C/N	N (%)	P (%)	K (%)	C (%)	Zn (mg kg^{-1})	Ni (mg kg^{-1})	Cu (mg kg^{-1})	Pb (mg kg^{-1})
PC	30.9±6.7	7.97±0.06	14.4±1.7	1.38±0.4	0.44±0.3	0.50±0.04	20±3.6	53.3±34.8	11.3±11.9	21.6±17.7	15.2±8.0
OMW [a]	-	4.91±0.39	31.2±1.87	0.08±0.004	0.03±0.003	0.27±0.01	2.50±0.15	3.80^b±0.22	6.54^b±0.65	2.02^b±0.12	1.53^b±0.12

[a] Average value of three years; letter b= mg l^{-1} (Zn, Ni, Cu and Pb of OMW).

Table 2. Effects of years and experimental treatments (OMW= Olive Mill Wastewater; OF= Organic-mineral Fertilizer; PC= Olive Pomace Compost, MV= Green manure of horse bean) on nitrate content (on dry weight basis) and SPAD.[a]

	NO_3^- mg kg^{-1}	SPAD
Year (Y)		
2006	55.8b	75.6a
2007	138a	76.5a
2008	125a	77.9a
	***	ns
Treatments (T)		
OMW	106a	76.1a
OF	106a	77.3a
PC	103a	75.9a
MV	110a	77.3a
	ns	ns
Y×T	ns	ns

[a] The values in the same column followed by different letters are significantly different at *P*≤ 0.05 (SNK). *, **, ***: Significant at *P*< 0.05, 0.01 and 0.001, respectively, ns= Not significant.

(2005; t0) and at the end (2008, tf) of the field trial in early December (at the end of the harvest). The samples were collected at the end of the projection of the tree canopy and between two trees. In particular, five samples were taken and then pooled in one sample at t0, being the previous fertilization management uniformly performed with green manure. Subsequently, the field was divided among fertilizer treatments, thus at tf stage a sampling with 3 repetitions for elementary plot was done. The samples were oven dried, ground to pass a 2-mm sieve and then analyzed for determining the following parameters: total N content (g kg^{-1}), by the Kjeldahl method; available Phosphorus (P_{av}; mg kg^{-1}), by using the ammonium molybdate-ascorbic acid method, according to the Olsen and Sommers method (Page *et al.,* 1982); exchangeable bases (K, Ca, Na and Mg; mg kg^{-1}) with the Page *et al.* (1982) methodologies and assayed by ICP-OES; Total Organic Carbon (TOC; g kg^{-1}), according to the Springer and Klee (1954) method; Total Extractable Carbon (TEC; g kg^{-1}) and humified organic carbon ((HA+FA)-C; g kg^{-1}) by the Sequi *et al.* (1986) procedure; soil mineral N [N-nitrate (NO_3^-)+N-ammonium (NH_4^+) exchangeable] extracted by 2M KCl (1:10, w/v), measured by continual flow colorimetry according to Krom (1980) and Henriksen and Selmer-Olsen (1970) for NH_4^+-N and NO_3^--N, respectively; Zinc (Zn), Copper (Cu), lead (Pb), and Nickel (Ni) (mg kg^{-1}), by ICP-OES after digestion in HNO_3 65% in a pressurized microwave.

Statistical Analysis

The statistical analysis was carried out using the General Linear Model Procedure (PROC GLM) of the SAS package (Sas Institute, 2012). The years were considered as a random effect, whereas fertilizer treatments were known as a fixed one. The differences among the experimental treatments were analyzed at the $P\leq$ 0.05 probability level, by applying the Student-Newman-Keuls (SNK) test. The differences found with the test for different main effect and interaction comparisons were calculated using the appropriate standard error term. Only the main effects of experimental treatments are presented in this paper, because the large part of the interactions were not significant. PROC BOXPLOT procedure was used to generate box plot for yield and pulp:pit ratio parameters in each treatment, thus providing quantitative information about these variables.

RESULTS

Characteristics of the Experimental Compost and Olive Mill Wastewater

Table 1 shows some important characteristics both of PC and OMW that were applied on the olive orchard in the three-year field trial. The pH value of PC was within the range of 6 to 8.5 allowed for mixed waste compost by Italian fertilizers legislation (Decree n. 75, 2010). As a consequence of a good level of total N, the C/N ratio was far below the threshold (< 25), whereas a C content in line with the threshold (≥ 20% on dry matter basis) was found. The contents of heavy metals in PC were all lower than the maximum values (i.e. Zn≤ 500 mg kg^{-1}; Ni≤ 100 mg kg^{-1}; Cu≤ 230 mg kg^{-1}; Pb≤ 140 mg kg^{-1}) established by the legislation in force. The OMW showed acidic pH, a high C/N ratio (due to the low N content) and very low levels of heavy metals.

Nitrate and SPAD Measurements and Chemical Composition of Leaves

No significant main effect of year was observed on SPAD readings (Table 2). Conversely, NO_3^-, P, and Ca contents showed significant differences. In particular, the lowest NO_3^- was observed in the first year as compared to the others. The P was lower in 2008 than in 2006 and 2007, whereas the highest Ca was found in 2006 and the other two years had lower and comparable values between them (data not shown). No significant year×treatment interactions were found. As regards to treatment effects, no significant differences were found for NO_3^- values and SPAD readings. By contrast, the analysis of chemical composition of leaves in each phenological stage (i.e., 1. Onset of vegetative growth; 2. Flowering and fruit setting; 3. Drupe formation, and 4. Pit-hardening) revealed some differences, as shown in Figure 1. In stage 1, the OF treatment determined the highest K content, whereas at stage 4, on the average of OF and PC, this nutrient was higher by 11 and 8.6% than OMW and MV, respectively. At stage 3 the MV determined a

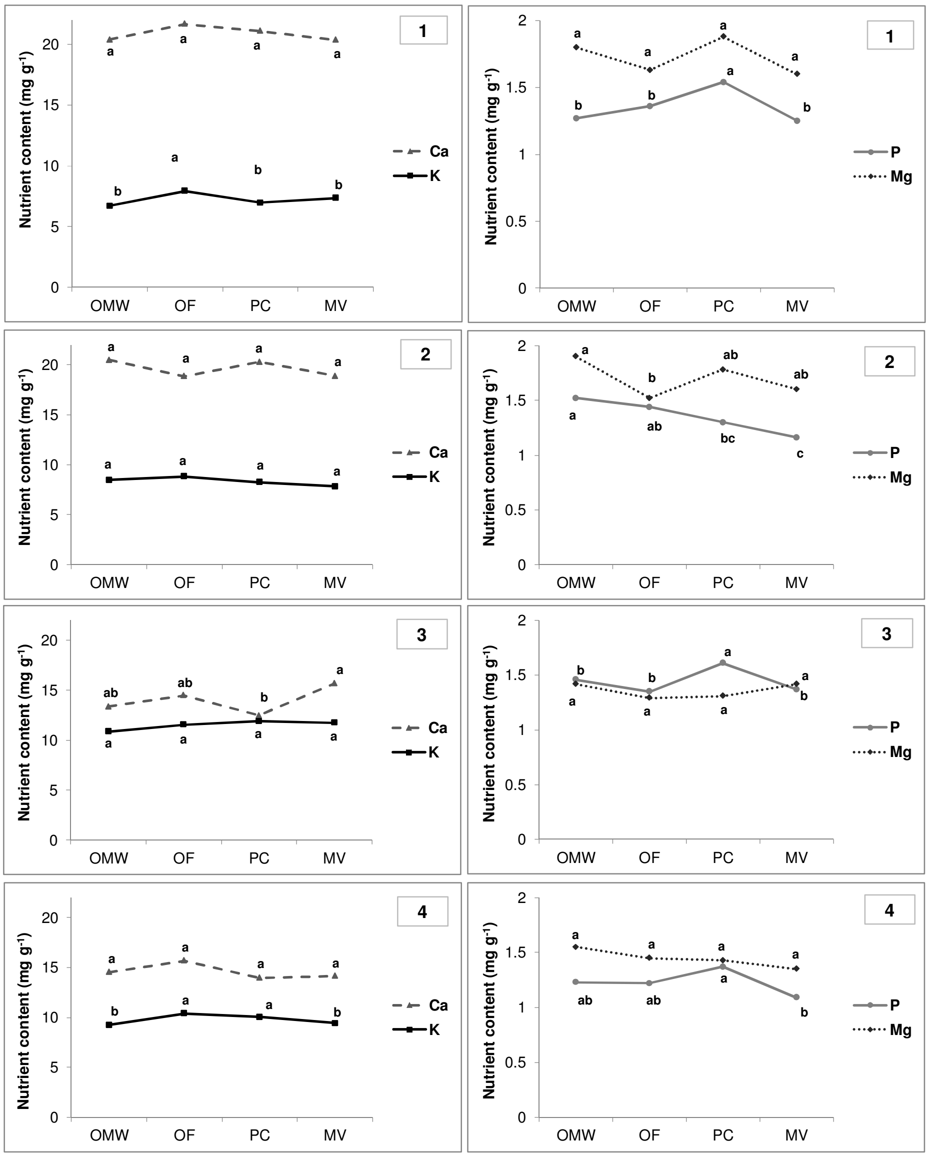

Figure 1. Effect of fertilizer treatments (OMW= Olive Mill Wastewater; OF= Organic-mineral Fertilizer; PC= Olive Pomace Compost, MV= Green manure of horse bean) on Ca and K leaves content, and P and Mg contents at each phenological stage (1. Onset of vegetative growth, 2. Flowering and fruit setting, 3. Drupe formation, and 4. Pit-hardening) in three years average. Within parameters, different letters indicate significantly different mean values according to SNK at $P \leq 0.05$ probability level.

Ca content significantly higher by 26% than PC but comparable to the other two treatments. The highest P value was observed for PC, as compared to the other treatments, both at stages 1 and 3. At stage 2, OMW determined a value comparable to OF for this parameter, but higher by 17 and 31% than PC and MV, respectively. At stage 4, no significant differences were found for PC with OMW and OF treatments. Finally, significant differences were found for Mg values between OMW and OF at stage 2, being the experimental treatment higher by 25% than the commercial one.

Effects of Fertilizer Treatments on Olive Tree Performance

Significant main effect of year was observed for olive yield, which was higher in 2007 as compared to the other years (Figure 2). In addition, both 2006 and 2008 presented a production below the minimum mean value recorded for the tested olive grove. The box plot for the yield in each treatment over the three-year trial, representing an overview of data distribution and variations, showed significant differences between MV and the other treatments (Figure 3). In particular, OMW and OF both presented a larger within-treatment variance and they showed the highest yield, which was on average significantly higher than MV and PC by 191 and 55%, respectively.

No significant year effect was observed on all the tested yield component parameters, unlike drupes oil production that showed the highest value in 2007 (Table 3). As for the treatment effect, most parameters, except pit weight and drupe length, showed significant differences. Due to the low yield under PC treatment, the fruit weight increased. In particular, the PC treatment had drupe and pulp weights higher than those for OF and MV, but comparable to OMW. Drupe weight was higher by 43% for PC than for the average of OF and MV, whereas PC showed pulp weight higher by 54% than the average of OF and MV treatments. Moreover, drupe width and pulp:pit ratio values were both the highest in PC, whereas no substantial differences were observed for these two parameters among the other treatments. Despite the observed positive results for olive yield components in PC, it showed lower drupes oil production than OMW and OF (by 33 and 36%, respectively) but higher than MV by 93%. No significant year×treatment interaction was found for yield and all the yield components. Among them it could be crucial to focus on pulp pit^{-1}, therefore, the box plot displayed in Figure 4 represents summary statistics. The interquartile range for MV, indicating how spread out the middle values are, better shows the significant difference with PC. The long

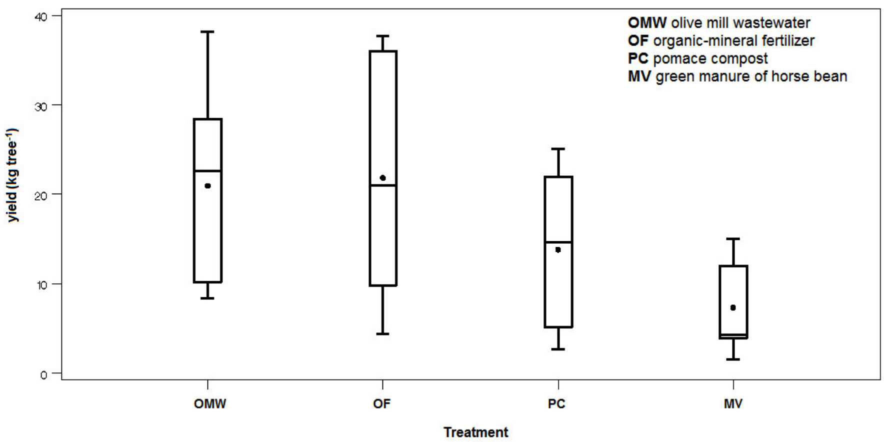

Figure 3. Box plot for yield (kg $tree^{-1}$) in each treatment, over the three-year period. Results were significant at $P \leq 0.001$ probability level.

Table 3. Effects of years and experimental treatments (OMW= Olive Mill Wastewater; OF= Organic-mineral Fertilizer; PC= Olive Pomace Compost, MV= Green manure of horse bean) on olive yield components and drupes oil production.[a]

	Drupe weight (g)	Pulp weight (g)	Pit weight (g)	Drupe length (mm)	Drupe width (mm)	Pulp pit^{-1}	Olive oil (kg tree^{-1})
Year (Y)							
2006	2.67a	2.03a	0.64a	20.3a	14.2a	3.16a	1.85b
2007	2.63a	1.99a	0.64a	20.3a	14.3a	3.04a	3.09a
2008	2.20a	1.63a	0.57a	19.1a	13.4a	2.84a	2.16b
	ns	ns	ns	ns	ns	ns	*
Treatments (T)							
OMW	2.49ab	1.88ab	0.61a	19.8a	13.9b	2.96b	3.09a
OF	2.14b	1.59b	0.54a	19.2a	13.2b	3.00b	3.24a
PC	3.14a	2.46a	0.68a	21.2a	15.3a	3.55a	2.07b
MV	2.24b	1.61b	0.62a	19.4a	13.4b	2.55b	1.07c
	*	*	ns	ns	*	**	***
Y×T	ns	ns	ns	ns	ns	ns	ns

[a] The values in the same column followed by different letters are significantly different at $P \leq 0.05$ (SNK).
*, **, ***: Significant at $P < 0.05$, 0.01 and 0.001, respectively, ns= Not significant.

upper whisker means that values are varied amongst the most positive quartile group. Finally, a broad overlap between the OMW, OF and PC distributions can also be observed.

Effects of Fertilization Strategies on Soil Parameters

Despite the different nutrient supplied by treatments (i.e., 640 g N 100 m^{-2} and 240 g P 100 m^{-2} by OMW; 900 g N 100 m^{-2} and 371 g P 100 m^{-2} by PC; 900 g N plant^{-1} and 314 g P plant^{-1} by OF and 645 g N 100 m^{-2} and 113 g P 100 m^{-2} by MV), the analysis of their effects on the soil chemical characteristics at the end (tf) of the three-year trial showed significant differences only for Ca content among macronutrients (Table 4). Anyway, the P_{av} value notably increased after repeated organic fertilizers application compared to t0. The PC and MV treatments determined the highest Ca content, while intermediate value was found for OF and the lowest one was in OMW. Treatments showed substantially decreasing and increasing Ca and Mg values, respectively, compared to t0.

No significant differences among treatments were observed for TOC and TEC parameters. Moreover, the TEC was substantially higher after fertilizers application than at the beginning of the experimental trial. The (HA+FA)-C also increased at tf in all the treatment plots (on average by 82%), and PC had the highest value (higher by 24% than MV), but comparable to that of OMW and OF treatment plots. No significant differences among treatments were found for NH_4^+-N and NO_3^--N, the latter being notably higher at tf than at t0.

As for the heavy metals content, the PC treatment had higher value of Zn than MV and OMW, but comparable to OF treatment. No significant differences among treatments were found for Cu, whereas the Ni content was higher (but comparable to MV) both in PC and OMW than in OF plot. Finally, the Pb content was particularly higher in PC than in OMW (by 33%) and MV (by 18%).

DISCUSSION

Effects of Fertilizer Treatments on Trees nutritional Status and Yield Performance

The leaf content of K, which is one of the most important minerals in olive nutrition (Erel *et al.,* 2013), fell below the sufficiency threshold of

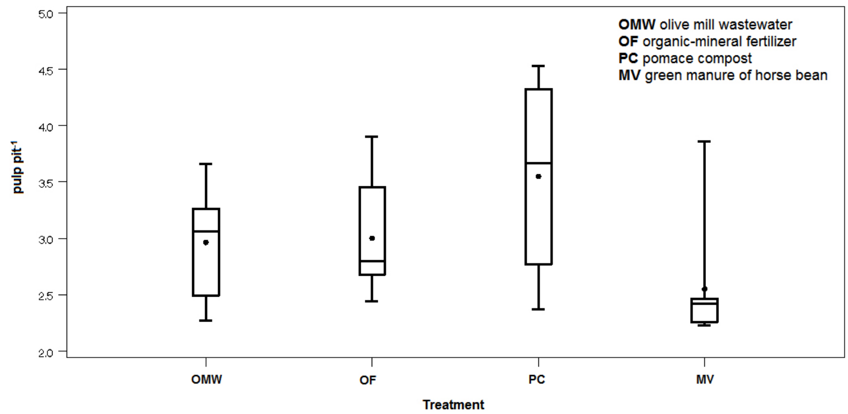

Figure 4. Box plot for pulp pit^{-1} in each treatment, over the three-year period. Results were significant at $P \leq 0.01$ probability level.

Table 4. Main soil parameters at the beginning (2005, t0) and at the end (2008, tf) of the field trial, divided by experimental treatments (OMW= Olive Mill Wastewater; OF= Organic-mineral Fertilizer; PC= Olive Pomace Compost, MV= Green manure of horse bean).[a]

Parameters[b]		t0	tf				
			OMW	OF	PC	MV	*F*
N tot	(g kg^{-1})	1.23±0.09	1.16a	1.14a	1.15a	1.12a	ns
P_{av} [b]	(mg kg^{-1})	9.87±0.60	29.5a	22.4a	24.2a	25.1a	ns
K_{ex} [c]	(mg kg^{-1})	834±66.7	1109a	963a	934a	977a	ns
Ca_{ex}	(mg kg^{-1})	4737±378	3823c	4082b	4554a	4449a	**
Na_{ex}	(mg kg^{-1})	231±23.2	35.7a	35.1a	40.7a	38.4a	ns
Mg_{ex}	(mg kg^{-1})	128±10.2	197a	181a	185a	187a	ns
TOC [d]	(g kg^{-1})	11.3±0.90	11.2a	11.7a	10.6a	11.1a	ns
TEC[e]	(g kg^{-1})	5.89±0.58	7.91a	7.75a	8.30a	7.57a	ns
(HA+FA)-C[f]	(g kg^{-1})	3.29±0.20	6.00ab	6.06ab	6.62a	5.34b	*
NH_4^+-N	(mg kg^{-1})	1.85±0.14	1.99a	1.97a	2.10a	2.08a	ns
NO_3^--N	(mg kg^{-1})	4.87±0.38	8.48a	8.06a	7.05a	7.98a	ns
Zn	(mg kg^{-1})	33.98±3.40	39.8bc	42.6ab	45.5a	38.0c	**
Cu	(mg kg^{-1})	13.38±0.80	15.0a	13.7a	14.5a	12.8a	ns
Ni	(mg kg^{-1})	20.99±1.67	22.7a	21.6b	22.9a	22.3ab	*
Pb	(mg kg^{-1})	7.91±0.47	7.75d	9.33b	10.3a	8.69c	***
pH		8.1±0.11	8.0	8.1	8.1	8.1	ns

[a] The values in the same column followed by different letters are significantly different at $P \leq 0.05$ (SNK). [b] Available; [c] exchangeable; [d] Total Organic Carbon; [e] Total Extracted Carbon, [f] Humified organic Carbon. *, **, ***: Significant at $P < 0.05$, 0.01 and 0.001, respectively, ns= Not significant.

0.8% presented by Fernández-Escobar (2010) only at the first phenological stage (Figure 1). At this stage, the OF treatment determined the highest K content. However, although the OF performance was consistent in the subsequent stages, the experimental treatments (particularly PC) showed similar effective outcomes. The P was the most variable nutrient, suggesting a better response to fertilization than the others. In particular, the best leaf P results in PC, at all phenological stages except the second one, could indicate a more effective release of this nutrient from composted materials during the cropping cycle, which can be matched with more favourable soil conditions. Conversely, the MV treatment determined significantly lower leaf P

[DOR: 20.1001.1.16807073.2016.18.7.4.4]

concentration in comparison with the other treatments in stage 2, which is one of the most important stages for olive production. This is probably as a consequence of the smaller P rate supplied with MV than with the other treatments (on average by 63%). It appeared to be a temporary result, being leaf P content from MV in the other stages comparable to OMW and OF, showing no evidence of P deficiency. Indeed, the comparable yield, drupes oil production and leaves composition results for OMW and OF treatments suggest their similar and suitable P supply. This indicates the possibility to replace OF with the wastewater. Although the acidic pH of OMW, likely due to the presence of organic acids and the degree of fruit ripening (Barbera *et al.,* 2013), did not negatively affect plant performance, mainly due to the pH buffering capacity of soil. Furthermore, this was probably a consequence of the application of relatively low doses, during the period when the trees were not actively growing, which could reduce the possibility for toxic effects. These findings were in accordance with those of Chartzoulakis *et al.* (2010) on a Cretan olive orchard. The OMW appeared to be an alternative to the MV, likely because, despite an equal N supply, OMW provided more P to trees than MV.

Under rainfed conditions as in the study area, the climate variations likely modified soil nutrients availability at the crucial growing stages. Indeed, the olive tree is widely known for its tendency for alternate bearing, which affects fruit yield in different years (Mert *et al.,* 2013). This behavior is a strategic mechanism, saving nutrient reserves for vegetative growth, to survive biotic and abiotic stresses. In spite of such evidence, as explained by Connell *et al.* (2002) fertilization regimes that improve the plant nutritional status may mitigate the alternate bearing cycle. It is necessary to note that the distribution of alternative organic materials to the soil, as in our research, generally shows positive effect after repeated applications. Then, testing the olive mill by-products in longer-term field experiments could reduce the biennial bearing cycle of the olive trees.

No differences were found among treatments for oil yield percent, but only among trial years. The oil yield was more influenced by the harvesting period, than by the treatments. Thus, the olive oil production accurately reflected the production of drupes. Although the lower yield and oil production results obtained for PC as compared to OMW and OF tratments, the values of olive yield components after compost application were all greater then those after green manure. Therefore, the MV might have determined the average yield reduction (Figure 2), whereas OMW and PC had an overall similar behavior than the OF. These results suggest that composts obtained by olive mill by-products, replacing the widespread fertilizing methods in organic farms, such as MV, are suitable to sustain olive orchard production (Toscano *et al.,* 2013). In addition, OMW and PC fertilizers had the highest comparable drupe and pulp weights, showing their analogous positive influence on olive yield components. The last results for PC were contradicted by olive oil production, unlike Toscano *et al.* (2013) findings. However, the broad overlap of the OMW, OF and PC distributions in the box plot of pulp:pit ratio suggests that differences among treatments could be interpreted only as a tendency, due to the large within-treatment variance (Figure 4). Therefore, despite the fact that the PC treatment had the highest mean value, the OMW, OF and PC seem to be all equally substitutable between them. In any case, from the point of view of drupes oil content, the PC appeared to be as ‘a second choice’ between experimental fertilizers, because OMW (as OF) determined higher value.

Effects of Fertilizer Treatments on Soil Fertility and Environment

No increase of total soil N from t0 to tf after OMW and PC applications was found, contrary to López-Piñeiro *et al.* (2006). In our investigated organic olive-grove, the alternative fertilizers from agro-food wastes determined total N values comparable to those of OF and MV plots. Furthermore, results regarding the effects on soil fertility highlighted that both the OMW and PC could be applied as fertilizers to sustain olive tree production, due to the possibility of supplying sufficient nutrients to plants. These results confirm the García-Ruiz *et al.* (2012) findings in similar Mediterranean conditions. On the other hand, the increase of P_{av} and exchangeable K contents of the soil at tf, as

[DOR: 20.1001.1.16807073.2016.18.7.4.4]

compared to t0, agreed with the results obtained by Montemurro *et al.* (2011) after repeated applications of OMW on other crops and confirms the findings of Fernández-Hernández *et al.* (2014) in olive grove. Similarly, Piotrowska *et al.* (2006) found an increase by 65% of available P content, in comparison with control at zero incubation time, due to the application of 80 m^3 ha^{-1} OMW. The low P content at t0 might be attributed to an effect of the previous fertilization strategy (i.e, repeated green manure) likely influenced by unknown biotic and abiotic factors. The P increase at tf was probably obtained for a positive cumulative effect in the MV plots, and for different reasons in the PC plots. In particular, according to Adler and Sikora (2003), adding compost to soil can increase water-extractable soil P by direct addition, dissolution or displacement of sorbed P, and reduction of sorption capacity for P. The observed increase in P values attributable to the organic fertilizers application, regardless of the P amount supplied, is also consistent with the results of other studies with different organic wastes (Paredes *et al.,* 2005). As highlighted by Ordoñez-Fernández *et al.* (2007), this increase can improve crop growth, but can also raise the concentration of dissolved P in surface runoff, leading to contamination of drinking water especially on olive groves more susceptible to soil loss. Therefore, it is important to define correct doses and time of application. On the other hand, at high pH like that in our study site (pH 8.1), soil P could become immobilized by large concentrations of calcium (as in the MV plots) and therefore, a portion of the added P becomes unavailable for plant uptake (Mengel and Kirkby, 1982), especially under prevalent dry conditions. In MV plots this outcome is supported by the lower leaf P contents during the cropping cycle (Figure 1) and it could be a further explanation for yield results. The low level of (HA+FA)-C after the MV application, likely reduced the possibility of forming soluble complexes with P by binding the Ca. Conversely, in PC plots the P immobilization could not occur, as demonstrated by both the fairly good nutritional status and yield outcomes maybe due to the higher (HA+FA)-C content in PC than in MV plots.

The C/N value of the on-farm PC produced in this research could be considered suitable for ready-to-use products, whereas the high C/N ratio of OMW is a value commonly found for this kind of by-product (Niaounakis and Halvadakis, 2006). The increasing values of TEC after PC (and also OMW) applications suggest that it is possible to substitute traditional methods for soil fertility improvement in organic olive groves. As discussed above, repeated applications of PC also significantly increased the (HA+FA)-C in comparison with MV and t0, whereas comparable values were found with OMW and OF plots. Anyway, Van-Camp *et al.,* (2004) suggested that PC could have longer lasting beneficial effects on soil physical and biological properties as compared to OF. It could be inferred that using PC is particularly important in semi-arid conditions, where the agricultural soils are subjected to degradation due to the high mineralization rate of organic matter (Diacono and Montemurro, 2010). Similar results were found by García-Ruiz *et al.* (2012) investigating the cumulative effects of composted olive pomace.

Inorganic soil N contents also increased with organic fertilizers application as compared to the t0, suggesting that mineral N had not been immobilized during the degradation of labile C constituents of organic materials. This is in agreement to that reported by López-Piñeiro *et al.* (2006). It can also be argued that it is possible to substitute MV with the experimental fertilizers, considering the comparable mineral N results of MV with PC and OMW ones at the end of the three-year experiment. The increased NO_3^--N after three years of organic fertilizers application indicated that a portion of the N, derived from the fertilizers could remain in the soil, although subject to losses in the rainfall occurrence, instead of being taken up by trees. In particular, the NO_3^--N, being minimally adsorbed by the soil particles, is highly susceptible to losses into ground and surface waters by infiltrating water, thus affecting the environment (Mengel and Kirkby, 1982). These residual N concentrations represent an important potential nutrient source and should pilot the fertilization program for the succeeding cropping cycles.

Finally, despite the low Zn, Cu and Pb contents in the experimental compost, an increase was found in soil concentration of these heavy metals after PC application at tf, probably due to accumulation effects. According to

Lakhdar *et al.* (2009) the presence of heavy metals in composts should not affect soil application in the short-term, because accumulation in soil occurs only after repeated applications on the same site. Moderate PC doses will not cause any risk of toxicity, and therefore application rates always have to be chosen on the basis of limited heavy metal loadings.

CONCLUSIONS

Raw (OMW) and treated (PC) olive mill wastes could play a fundamental role in organic olive tree ecosystems. Our findings suggested that, in organic farming, these alternative organic fertilizers/amendments could substitute traditional methods of soil fertility improvement for olive groves, without determining detrimental effects on soil parameters and olive yields. Also, the use of these by-products might represent a feasible solution for their recycling, thus solving the disposal problem, and closing the natural cycle of residues-resources particularly in organic horticultural production. The research outcomes further suggest that organic producers should take into account a system approach for olive grove fertilizing, to avoid the accumulation of soil mineral N, thus reducing the risk of leaching in the subsequent growing seasons. Our study represents a contribution to step up the knowledge on the olive mill waste recycling in organic olive orchards, but further studies should be encouraged in other sites under Mediterranean conditions, also to assess the possible long-term effects.

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کاربرد محصولات جانبی آسیاب زیتون : عملکرد محصول باغ زیتون ارگانیک و حاصلخیزی خاک

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چکیده

تجمع فصلی و دفع نادرست محصولات جانبی آسیاب زیتون ممکن است برای محیط زیست مضر باشد. درمقابل، بازیافت مناسب آن ها به عنوان اصلاح خاک می تواند یک راهکار مناسب باشد. بنابراین، هدف این مطالعه سه ساله در مزرعه: ۱) مطالعه اثرات پساب آسیاب زیتون (OMW) و کمپوست تفاله زیتون (PC) بر وضعیت تغذیه گیاه و عملکرد بیشه زیتون ارگانیک ۲) تاثیر در ویژگی های اصلی خاک ۳) تایید این که کودهای تجربی می توانند جایگزین عملیات های گسترده حاصلخیزی خاک شوند یا نه می باشد. تیمارهای OMW و PC با کودهای تجاری آلی-معدنی (OF) و کود سبزhorse bean (MV) مقایسه شد. وضعیت تغذیه گیاه ، خواص خاک و عملکرد زراعی درمان ها بررسی شد. میانگین بازده OMW و OF به طور معنی داری بالاتر از MV و PC بود (به ترتیب ۱۹۱٪ و ۵۵٪). برگ حاوی فسفر در PC نشانگر انتشار موثرتر موادغذایی در مقایسه با بقیه تیمارها می باشد، که می تواند با شرایط مطلوب تر خاک همسان باشد. عملکرد و محتویات برگ در OMW و OF بیان میکند که بهتر است OF با OMVجایگزین شود. تاثیرات بر تولید روغن زیتون و حاصلخیزی خاک نشان می دهدکه OMW (و PC به عنوان دومین انتخاب) می تواند برای حفظ تولیدات درخت زیتون استفاده شود و جایگزین کودهای سنتی شود.