LONG-TERM EFFECTS OF TWO-PHASE OLIVE MILL WASTE ON BIOCHEMICAL PROPERTIES OF AN OLIVE GROVE SOIL

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1 INTRODUCTION

The olive mill extraction of oil is a widespread industry in Mediterranean countries, generating large amounts of olive mill waste over a limited time period. The new technology for olive oil extraction is a continuous centrifuge two-phase process that in Spain generates more than 4 000 000 Mg yr^{-1} of two-phase olive mill waste (TPOMW), which has to be suitably managed in order to avoid the associated environmental impact. Soils in many Mediterranean areas are generally characterized by low organic matter content, which greatly contributes to their limited fertility and production levels, and their great exposure to degradation processes such as erosion (Albaladejo et al., 1994).

Since TPOMW contains large amounts of organic matter, application of TPOMW to soils may play a fundamental role in the maintenance of the olive tree ecosystem and close the residue-resource gap (Roig et al., 2006; López-Piñeiro et al., 2008). Several studies have investigated the use of composted TPOMW as a soil amendment, although most were carried out under short-term and/or greenhouse conditions (e.g., Altieri and Exposito, 2008; Fornes et al., 2009). However, very few studies concerning the effect of direct (not composted) application of TPOMW as organic amendment have been carried out, although this can mean important advantages in terms of time and costs. These studies have demonstrated the beneficial effects of this raw waste in restoring crop productivity on degraded soils (López-Piñeiro et al., 2006 and 2008). Little is know, however, of the long-term impact of raw TPOMW on soil enzyme activities, although these parameters would give helpful information concerning functionality and productivity because of their central role in nutrient cycling and their sensivity to management (Moreno et al., 2009).

The aim of the present study was to evaluate the cumulative effects of eight years of repeated applications of TPOMW on the biochemical properties (dehydrogenase, β-glucosidase, urease, phosphatase, and arylsulphatase activities) of a typical Mediterranean olive grove soil. The influence on biochemical properties of organic matter transformation after two years of the TPOMW's natural biodegradation in the soil was also assessed.

2 MATERIALS AND METHODS

A field experiment was conducted in Elvas (Portugal) on a representative olive grove (*Olea europaea* L.) whose soil was left unamended or amended annually for eight years with two-phase olive mill waste. The soil, classified as a Cutanic Luvisol (ISSS-ISRIC-FAO 1994), consisted of 19.7% clay, 19.7% silt, and 60.6% sand. The fresh TPOMW was obtained from an oil industry which employs a two-phase decanter centrifugation system. The main characteristics of the soil and the two-phase olive mill waste are given in Table 1. The experimental design consisted of nine plots in the olive grove, with amendments made in a complete randomized design with three replicates per treatment. Each plot consisted of 4 × 3 trees. The three treatments were: 30 (T30) and 60 (T60) Mg ha^{-1} of two-phase olive mill waste, dry weight equivalent, and unamended (T0) treatment. Amendments were applied annually in February (from 1999 to 2006), manually spreading the waste on the soil surface, followed by arable-level homogenization using a mould-board plough. Soil subsamples (0-25 cm depth) from each olive grove plot were taken randomly in December 2006 and 2008. Moist and air-dried samples were passed through a 2-mm sieve and stored at 4 °C until use. Measurements made in 2006 and 2008 represented the "cumulative" (T0C, T30C, and T60C samples) and "residual" (T0R, T30R, and T60R samples) effects, respectively.

TABLE 1 **Selected characteristics of the soil and two-phase olive mill waste**

	Organic Carbon	**pH**	**Total N**	**Electrical conductivity**	**P**	**K**	**Water soluble phenols**
Unit	(g kg^{-1})		(g kg^{-1})	(dS m^{-1})	(g kg^{-1})	(g kg^{-1})	(g kg^{-1})
Soil	13.3	8.10	1.49	0.513	0.013[a]	0.200[a]	0.016
TPOMW	535	5.70	16.0	5.02	2.75[b]	15.0[b]	7.3

[a]Available P and K; [b]Total P and K

Dehydrogenase (DH) activity was determined by the method of Trevors (1984) modified by García et al. (1993). One gram of soil was incubated for 20 h at 20 °C in the dark with 0.2 ml of 0.4% 2-*p*-iodophenyl-3 *p*-nitrophenyl-5 tetrazolium chloride (INT) as substrate. At the end of the incubation the iodonitrotetrazolium formazan produced was extracted with 10 ml of methanol and the absorbance measured at 490 nm. To assay urease (UR) activity, 2 ml of 0.1 M pH 7.0 phosphate buffer and 0.5 ml of 1.066 M urea were added to 0.5 g of soil and incubated for 1.5 h at 30 °C. The ammonium released in the hydrolytic reaction was measured spectrophotometrically at 636 nm (Kandeler and Gerber, 1988). The activities of β-glucosidase (GLU), phosphatase (PHO), and arylsulphatase (ARS) were determined by incubating 1 g of soil with 4 ml of 25 mM 4-nitrophenyl-β-d-glucanopyranoside in 0.1 M modified universal buffer (MUB) pH 6.0, MUB pH 11, and 5 mM 4-nitrophenyl sulphate in 0.5 M acetate buffer pH 5.8 for 1h at 37 °C (Tabatabai, 1982, Tabatabai and Bremmer, 1969, and Tabatabai and Bremmer, 1970, respectively). The samples were then cooled to 2 °C for 15 min to stop the reaction, and the *p*-nitrophenol produced in the enzymatic reactions was determined at 400 nm, 398 nm, and 410 nm for GLU, PHO, and ARS, respectively. Blank assays without soil and with substrate were made at the same time as controls

3 RESULTS AND DISCUSSION

The DH activity increased significantly after application of both TPOMW rates (Table 2). Compared to the control, DH activity increased by about 70% and 115% in the cumulative year and by about 29% and 49% in the residual year, at the lower and higher rates, respectively. These results suggest that the addition of a readily available C source from TPOMW could promote the growth of indigenous microorganisms, and therefore result in increased synthesis of DH. However, although DH activity was always greater in amended than in unamended soils, even in the amended soils it decreased, significantly at the higher rate, two years after the last TPOMW application, probably because most of the readily available organic matter had decomposed, with the humified organic matter being more resistant to microbial mineralization. Similar results are reported by Piotrowska et al. (2006), although in laboratory experiments and using a three-phase olive mill waste water, and by García-Gil et al. (2000) in a field study but using municipal solid waste compost.

The GLU activity also increased after TPOMW addition and this effect was more evident with increasing amendment rate (Table. 2). This could indicate the soil's achieving the capacity to utilize the carbohydrate material added with TPOMW (Piotrowska et al., 2006). Furthermore, as GLU is mainly produced by fungi (Perucci, 1992), its increased activity suggests that the presence of TPOMW caused a shift in the relative proportions of fungi and bacteria, especially in the residual year in which a significant increase in electric conductivity was also observed (data not shown).

In the cumulative and residual years of the experiment, the TPOMW application greatly increased the urease (UR) activity (Table 2) compared with the control, although the residual effect was less pronounced than the cumulative effect. In particular, the UR activity increase over the unamended soil was by factors of 4.8 and 3.4 at 60 Mg ha^{-1} for the cumulative and residual years, respectively. The high concentration of N, the high level of available substrate, and the demand for nutrients by vegetation or microorganisms could lead to a high activity of these enzymes during the mineralization of TPOMW (García-Gil et al., 2000; Fernández et al., 2009). The UR increase was similar to those reported by Fernández et al. (2009) in short-term study where a Mediterranean soil was amended with two kinds of sewage sludge. However, in a laboratory study using olive mill waste water amended soils (Piotrowska et al., 2006), the urease in the amended soils increased much less than in our study, probably because the N applied was also much lower since the amendment was only added once, not repetitively for years as in our experiment. Similarly to UR, compared to the control the phosphatase (PHO) activity increased by factors of 2.7 and 2.8 at 60 Mg ha^{-1} for the cumulative and residual years, respectively (Table 2).

TABLE 2 **Cumulative and residual effects on the selected soil enzyme activities after repeated application of the two-phase olive mill waste**

	Dehydrogenase (mg INTF g^{-1} h^{-1})	**β– glucosidase (μmol pNP g^{-1} h^{-1})**	**Urease (μg NH_4^+ g^{-1} h^{-1})**	**Phosphatase (μmol pNP g^{-1} h^{-1})**	**Arylsulphatase (μg pNP g^{-1} h^{-1})**
2006					
T0C	1.02a ± 0.01	2.20a ± 0.05	14.9a ± 0.5	1.25a ± 0.09	15.9a ± 1.6
T30C	1.74b ± 0.02	5.33b ± 0.47	56.5b ± 3.9	2.72b ± 0.15	35.6b ± 0.4
T60C	2.20c ± 0.04	5.90b ± 0.49	72.2c ± 3.9	3.43c ± 0.12	34.8b ± 1.1
2008					
T0R	1.30a ± 0.02	2.35a ± 0.12	17.9a ± 0.3	1.02a ± 0.02	15.9a ± 0.6
T30R	1.67b ± 0.03	6.25b ± 0.32	43.3b ± 0.8	2.55b ± 0.06	52.3b ± 1.9
T60R	1.94c ± 0.03	6.66b ± 0.17	61.0b ± 2.3	2.87b ± 0.09	54.1b ± 0.8

Values with the same letter within a column, for a given sampling date, are not significantly different at $p<0.05$ level of probability.

A significant increase in arylsulphatase (ARS) was also observed in TPOMW amended soils in the cumulative and residual years, independently of the loading rate of the applied waste (Table 2). The residual effect was more pronounced than the cumulative effect. Compared with the control, ARS increased by about 120% and 240% at the greater rate of TPOMW application in the cumulative and residual years, respectively. This seems to indicate that the nature of the organic matter has be to taken into account, since not all organic matter has the same effectiveness for ARS activity. Indeed, ARS activity correlated positively and highly significantly ($P<0.001$) with HA and PG ($r=0.891$, 0.790), and significantly ($P<0.01$) with TOC ($r=0.760$) (data not shown).

4 CONCLUSIONS

A long-term history of field amendment of soils with two-phase olive mill waste greatly influenced the soil enzyme activities. The TPOMW soil application increased dehydrogenase, urease β-glucosidase, phosphatase, and arylsulphatase activities of the soils with increasing TPOMW rate, but also increasing the humified organic matter fraction. Since these biochemical indicators are believed to be essential for the sustainability of organic-farming systems, the results of this study reveal that recycling of fresh TPOMW back to the olive grove as an organic amendment may be considered a good strategy both for its disposal and for the restoration of frequently degraded olive grove soils.

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