pagepress

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In situ olive mill residual co-composting for soil organic fertility restoration and by-product sustainable reuse

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Abstract

The addition of organic matter in the form of compost improves overall physical, chemical and biological properties of soils but, to be really sustainable, the composting process should be carried out using the by-products available *in situ*. Two different soils of a Mediterranean olive orchard, one managed traditionally (NAS) and the other amended with compost (AS), were investigated in a two-year experiment. Increases in total organic matter, total nitrogen and pH, were detected in AS if compared to NAS. Significant increases in total and specific microbial counts were observed in AS, with a clear amelioration of microbiological soil quality. The results demonstrated that soil amendment using compost deriving from olive mill by-products can be an important agricultural practice for supporting and stimulating soil microorganisms and, at the same time, for re-using these by-products, so avoiding their negative environmental impact.

Introduction

The indiscriminate use of agro-chemicals, the excessive irrigation and soil tillage often lead to soil degradation, particularly in Mediterranean areas as result of losses of organic matter and desertification (Durán Zuazo and Rodríguez Pleguezuelo, 2008). Environmental quality and productivity of agro-ecosystems, also synthetically defined as *soil fertility* (Abbott and Murphy, 2007), have emphasized the need to develop management practices that maintain soil resources (Saviozzi *et al*., 2002). One of these practices is the addition of organic matter in the form of compost to improve overall physical, chemical and biological properties of soils. The main problem is that compost is often not easily available (*e.g.,* distance from the field) nor economically convenient (*e.g.,* transport and production costs), making its application in field not always realistic. Therefore, the composting process should be carried out using the by-products available *in situ*, obtained with non-industrial procedures based on spontaneous degradation processes.

The olive pomace (OP) is the residue of the first oil extraction from olives (crude olive cake) and is usually used as an organic amendant for olive grove or other crops soils (Alburquerque *et al*, 2004). In terms of agronomic value, OP watered with olive mill wastewater (OMWW), another abundant by-product deriving from olive milling, or with other organic material, lead to a product that supplies nutrients to plants and is an efficient method for the disposal of olive mill residuals (Hachicha *et al*., 2008; Sellami *et al*., 2008). OP derives from ground olive stones and pulp and has an average moisture of 8-10% (Alburquerque *et al*., 2004). On the other hand, OMWW is composed by olive vegetation water and the water used in the different stages of olive oil production, and it constitutes an environmental emergency due to its considerable polluting organic load (Niaounakis and Halvadakis, 2006). As OMWW does not generally contain sufficient N and P for an adequate aerobic purification process, its degradation should be performed by co-composting procedures (Paredes *et al*., 2002; Fadil *et al*., 2003). For this reason, both OP and olive branches and leaves deriving from pruning residues (OPR) are often added to the composting matrices containing OMWW (Paredes *et al*., 2002).

The capacity of a soil to function in a productive and sustained manner is strongly dependent on the activity and diversity of microbial communities, that play a key role in pedogenetic processes and in nutrient turnover and availability to the plants (Joshi *et al*., 2009). For this reason, microbial abundance and diversity can function as bio-indicators of soil quality. On this basis, the aim of this work was to compare two different soils of a Mediterranean olive orchard, one managed traditionally and the other amended *in situ*. We hypothesized that soil fertility could be ameliorated with the application in field of olive mill aerobically stabilized by-products.

Materials and methods

Study site and compost maturation

The study was carried out in a 15-year-old olive orchard (*Olea europaea* L.; cv. *Nocellara* and cv. *Nocellara* messinese, planted at a

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Key words: compost, *Olea europaea* L., olive mill wastewater, olive pomace, pruning residues, soil microbial fertility.

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distance of 6 m x 4 m) located in southern Italy (San Demetrio Corone, Calabria Region, 39°34'0" N; 16°21'0" E).

The matrix for compost production (maturation from March to September) included: olive pomace (OP) and olive mill wastewater (OMWW) deriving from a three-phases olive mill, and rain water, added with grinded olive branches and leaves deriving from olive pruning residues (OPR). Depending on the by-products availability in the experimental field, the matrix composition in the three years was the following: 30 t OP + 8 t OPR in 2007; and 40 t OP + 5 m^3 OMWW + 25 t OPR in 2008.

The matrix, uniformly mixed to form a trapezoidal parallelepiped pile (volume = 60 and 129 m^3 in 2007 and 2008, respectively) placed outdoor in open field, resulted to have a semi-solid consistency. It was blended with a mechanical shovel every 7 days, in order to ensure the aeration (to reduce anaerobic fermentation) and control biomass warmth, and was subjected to a spontaneous degradation by autochthonous microorganisms. At the end of the composting process, 5-kg compost samples (n=20) were randomly collected in different parts and depths of the pile, placed in sterile plastic bags, and stored in a refrigerated box at 4°C. On these compost samples, the mean chemical parameters were measured (Table 1).

In November of both 2007 and 2008, the compost was uniformly spread in a half of the olive orchard over a rate of 60 t ha^{-1} (150 kg $tree^{-1}$), and buried with a light disk-arrow tilling (amended soils; AS), whereas in the other half of the orchard, compost was not added (non amended soils; NAS).

Soil chemical and microbial analysis

In June and October 2008 and 2009, five composite samples of bulk soil (10 five-cm-diameter cores pooled on site) were collected from surface soils (0-20 cm) along the inter-rows for both AS and NAS treatments. The sites at borderlines between the two treatments were avoided. After removing visible crop residues, soil samples were immediately stored in sterilized plastic pots at 4°C until the following chemical and microbiological. For soil chemical analysis, organic carbon content (Corg) in soil samples, was determined by Walldey-Black procedure based on soil oxidation with excess $Cr_2O_2^{7-}$ in H_2SO_4, whereas total nitrogen (N) was determined by Kjeldahl method (Violante, 2000). For the measurement of soil pH, a soil aliquot of 10 g was added to 50 mL of distilled water, the container was shaken for 3 min, and the soil was left to settle for 2 min (Violante, 2000). For soil microbiological analysis, Three replicates of 100 g-sub-samples (dry weight equivalent) of each soil sample were suspended in 900 mL sterile 0.1% sodium pyrophosphate-one quarter strength Ringer solution (NaCl 2.25 g L^{-1}, KCl 0.105 g L^{-1}, $CaCl_2$ 0.045 g L^{-1}, $NaHCO_3$ 0.05 g L^{-1}, and citric acid 0.034 g L^{-1}) and sonicated for 2 min to disperse microbial cells. Ten-fold serial dilutions of the supernatants were made in sterile one-quarter strength Ringer solution. Diluitions were prepared to obtain colony-forming units (CFU) counts in the range of 30-300 per plate. All the microbial groups investigated were assessed in triplicate through plate-counter and expressed as CFU per mL, except for Azotobacter.

One hundred µL aliquots of the corresponding decimal dilution were spread-plated on 1/10 strength TSA (Tryptic Soy Agar) medium (Oxoid Lim., Hampshire, UK) amended with 0.1 mg mL^{-1} cycloheximide (Sigma, Medina, NY, USA) for total cultural bacteria, and inoculated in MEA (Malt Extract Agar) medium containing 0.03 mg mL-1 streptomycin and 0.02 mg mL-1 tetracycline (Sigma) for total fungi. Counting took place after suitable incubation period (72 h for bacteria and 120 h for fungi) at 28°C.

Actinomycetes were isolated by using Casein Starch Agar (Oxoid) supplemented with 0.12 mg mL^{-1} cycloehximide (Sigma). *Pseudomonas* was cultured on *Pseudomonas* Agar Base medium (Oxoid) with the addition of *Pseudomonas* C-N Supplement (Oxoid). The isolation of *Bacillus* was carried out in PCA (Plate Count Agar) nutritive medium (Oxoid) supplemented with 0.20 g K_2HPO_4 and 0.05 g KH_2PO_4, and was performed in diluted suspensions placed in water-bath at 80°C for 10 min, in order to kill termolable non-*Bacillus* microorganisms. Azotobacter was quantified using Brown's substrate (Oxoid), whereas proteolytic bacteria were quantified by the MPN method in a cultural medium containing gelatine (Oxoid). *Pseudomonas* was cultured on Pseudomonas Agar Base medium (Oxoid) with the addition of *Pseudomonas* C-N Supplement (Oxoid).

Statistical analysis was carried out using the Sigmastat 3.1 sofware (SPSS Inc., Chicago, IL, USA). One-way analysis of variance (ANOVA) of soil chemical and microbial parameters was performed with orchard management (AS and NAS) as factor. Means were separated according to Duncan's multiple comparison test at $P<0.05$ (n=5).

Results and discussion

Turnover of soil organic matter is chiefly carried out by fungi, actinomycetes, and bacteria, major decomposers of complex polymers such as lignocelluloses and chitin (Govaerts *et al*., 2008). Simultaneous

Table 1. Compost chemical parameters (mean values 2007-2008) measured at the end of composting process. Each value represents the mean (n=20) ± standard deviation.

Parameter	Unit of measure	Value
pH	-	7.05±0.35
Conductivity	S cm^{-1}	854±39.0
Total organic carbon	%	31.4±0.99
Organic matter	%	54±1.70
Total nitrogen	%	1.3±0.16
P_2O_5	%	0.7±0.03
K_2O	%	1.15±0.10
Sodium	mg L^{-1}	118.1±14.0
Magnesium	mg L^{-1}	14.6±0.9
Calcium	mg L^{-1}	62.5±8.8
Boron	mg L^{-1}	1.0±0.2

Table 2. Soil organic matter, total nitrogen and pH in compost-amended and non amended (soils measured during the experimental period. Each value represents the mean (n=5) ± standard deviation.

Sampling time	Treatment	$C_{or}g$ (% of dry soil)	N (mg g^{-1} of dry soil)	pH
June 2008	AS	1.22±0.12^b	2.0±0.1^b	7.6±0.1^a
	NAS	0.88±0.21^c	1.5±0.4^c	7.9±0.4^a
October 2008	AS	1.42±0.18^a	2.2±0.3^b	7.3±0.3^b
	NAS	0.91±0.27^c	1.7±0.1^c	8.0±0.2^a
June 2009	AS	1.50±0.30^a	2.4±0.4^a	7.2±0.2^b
	NAS	0.93±0.11^c	1.4±0.2^c	7.9±0.3^a
October 2009	AS	1.62±0.34^a	2.5±0.2^a	7.2±0.2^b
	NAS	0.94±0.16^c	1.7±0.2^c	8.0±0.3^a

C_{org}, soil organic matter; N, nitrogen; AS, compost-amended soil; NAS, non amended soil; ^{a,b,c}values followed by a different letter are significantly different at P≤0.05, according to Duncan's mean separation test.Corg, soil organic matter; N, nitrogen; AS, compost-amended soil; NAS, non amended soil; a,b,cvalues followed by a different letter are significantly different at P≤0.05, according to Duncan's mean separation test.

increases in total organic matter, total nitrogen and pH, were detected in AS, if compared to NAS (Table 2). The content of soil organic matter in 2008 passed from 1.22% in June 2008 to 1.42% in October 2008, reaching values of 1.50 and 1.62% in June and October 2009, respectively (Table 2), with an increase of 72% during the whole experimental period if compared with NAS. The values of pH in NAS remained stable throughout the experimental period, with no significant differences between spring and autumn periods (Table 2). Starting from the second sampling date, AS showed significantly lower values of pH if compared to NAS (Table 2). This was likely due to the digestion of soil organic matter by microorganisms, particularly *Pseudomonas* and *Bacillus*, with the consequent release of organic and carbonic acids, as demonstrated by other authors (Singh and Amberger, 1998; Misra *et al*., 2003) and supported by the observed continue increase in *Pseudomonas* and *Bacillus* number during the experiment (Figure 1).

Significant increases in total and specific microbial counts were observed in AS, with a clear amelioration of microbiological soil quality if compared to NAS (Figure 1). Particularly, the different soil treatments significantly affected both total cultivable bacteria and total fungi, whose numbers were significantly lower in NAS (Figure 1A). In AS, the trends of both these counts increased during the experimental period if compared to NAS (Figure 1A). Total cultural bacterial counts in AS did not significantly differ in the last three sampling dates, so suggesting that total bacterial growth was not inhibited by the presence of OMWW (applied only in 2009), a by-product with a high concentration polyphenols having a possible anti-microbial action (Alburquerque *et al*., 2004; Niaounakis and Halvadakis, 2006; Hachicha *et al*., 2008). By contrary, total fungi did not show an inhibition due to OMWW, as they constantly increased during the experimental period and their highest value (9.51 log CFU g^{-1} soil) was observed in the last sampling date (Figure 1A). Actinomycetes and fungi reached levels of 10.17 and 9.51 log CFU g^{-1} soil, respectively, at the end of the experimental period (Figure 1A). These two groups play a major role in improving soil fertility, as they produce a number of enzymes that help degrade organic plant material, are abundant in soils rich of organic matter, and are able to use root exudates as carbon source, supplying roots with easily assimilable nitrates (Sofo *et al*., 2010).

In all the sampling dates, the numbers of actinomycetes, *Pseudomonas*, *Bacillus* and *Azotobacter* were significantly higher in AS than in NAS and they increased with time (Figure 1). Moreover, for actinomycetes, *Pseudomonas* and *Azotobacter*, significant differences were found between the spring and autumn sampling periods, with higher values in June (Figure 1). These differences were likely due to the sensitivity of these microbial groups to soil temperature, whereas Bacillus were not affected by seasonability as this genus mainly includes euriterm bacterial species and strains (Giri *et al*., 2005).

In October 2009, *Pseudomonas* and *Bacillus* in AS reached levels of 9.96 and 8.23 log CFU g^{-1} soil, respectively, whereas remained stable in NAS (Figure 1B). These two microbial groups are the main decomposers of complex polymers, such as lignocelluloses and chitin, but they also have proteolytic enzymes and are of key-importance important for the production of assimilable nitrogen in soils (Zaitlin *et al*., 2004; Brady and Weil, 2008). The observed high number of *Pseudomonas* and the significant differences in their number between AS and NAS (Figure 1) indicates that these microorganisms were likely affected by the organic content without being particularly inhibited by OMWW, as also found by Sofo *et al.* (2010) in soils of a sustainably managed olive orchard. Higher inputs of organic matter in AS caused a significant increase in the microorganisms responsible for nitrogen fixation (*Azotobacter*) (Figure 1), that can be partially responsible for the significant increases in soil total nitrogen of AS (Table 2). This information is of particular importance for semi-arid environments, where soil N is often limiting for crop growth due to the high rates of soil mineralization, nutrients leaching and soil erosion (Gruhn *et al*., 2000; Casacchia *et al*., 2010).

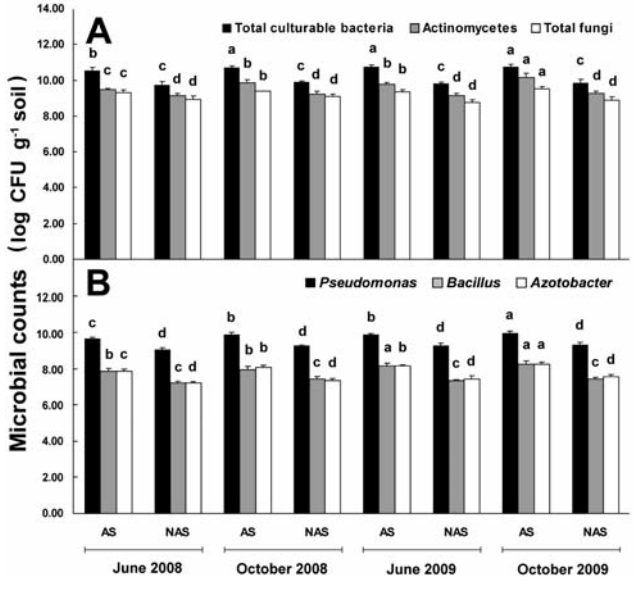

Figure 1. A) Counts of total cultural bacterial (black columns), actinomycetes (grey), and total fungi (white) in compost-amended (AS) and non amended (NAS) soils, measured during the experimental period; B) Counts of Pseudomonas (black columns), Bacillus (grey), and Azotobacter (white) in compost-amended (AS) and non amended (NAS) soils, measured during the experimental period. For each microbial group, values with different letters are significantly different at P≤0.05, according to Duncan's mean separation test.

Conclusions

Soil amendment using compost deriving from olive mill by-products, produced and applied *in situ*, can be an important agricultural practice for supporting and stimulating soil microorganisms, that in turn influence soil fertility and plant growth by increasing nutrients availability and turnover (Gruhn *et al*., 2000; Borken *et al*., 2002; Govaerts *et al*., 2008; Joshi *et al*., 2009). At the same time, the co-composting procedure here used can be a reliable and sustainable way to re-use these by-products, so avoiding their negative environmental impact. On this basis, *in situ* olive mill residual co-composting, together with conservation tillage, cover crops adoption, and adequate irrigation, fertilization and pruning (Sofo *et al*., 2010), can be included within the sustainable agronomic practices that can be adopted in Mediterranean orchards to improve soil fertility while maintaining top yields of high quality.

References

Abbott LK, Murphy DV, 2007. Soil biological fertility: a key to sustainable land use in agriculture. Springer Ed., New York, NY, USA, pp 132-150.

Alburquerque JA, Gonzálvez J, García D, Cegarra J, 2004. Agrochemical characterisation of "Alperujo", a solid by-product of the two-phase centrifugation method for olive oil extraction. Bioresource Technol. 91:195-200.

Borken W, Muhs A, Beese F, 2002. Changes in microbial and soil properties following compost treatment of degraded temperate forest soils. Soil Biol. Biochem. 34:403-12.

Brady NC, Weil RR, 2008. Elements of the nature and properties of soils. Pearson Prentice Hall Ed., NJ, USA.

Casacchia T, Briccoli Bati C, Sofo A, Dichio B, Motta F, Xiloyannis C, 2010. Long-term consequences of tillage, organic amendments, residue management and localized irrigation on selected soil micro-flora groups in a Mediterranean apricot orchard. Acta Hortic. 862:447-52.

Durán Zuazo VH, Rodríguez Pleguezuelo CR, 2008. Soil-erosion and runoff prevention by plant covers. A review. Agron. Sustain. Dev. 28:65-86.

Fadil K, Chahlaoui A, Ouahbi A, Zaid A, Borja R, 2003. Aerobic biodegradation and detoxification of wastewaters from the olive oil industry. Int. Biodeter. Biodegr. 51:37-41.

Giri B, Giang PH, Kumari R, Prasad R, Varma A, 2005. Microbial diversity in soils. In: F. Buscot and A. Varma (eds.) Microorganisms in soils: roles in genesis and functions. Soil Biology, vol.3. Springer-Verlag ed., Berlin, Germany, pp 19-55.

Govaerts B, Mezzalama M, Sayre KD, Crossa J, Lichter K, Troch V, Vanherck V, de Corte P, Deckers J, 2008. Long-term consequences of tillage, residue management, and crop rotation on selected soil micro-flora groups in the subtropical highlands. Appl. Soil Ecol. 38:197-210.

Gruhn P, Goletti F, Yudelman M, 2000. Integrated nutrient management, soil fertility, and sustainable agriculture: current issues and future challenges. Food, Agriculture, and the Environment Discussion Paper 32. Int. Food Policy Research Inst. Publ, Washington, DC, USA.

Hachicha S, Sallemi F, Medhioub K, Hachicha R., Ammar E. 2008. Quality assessment of composts prepared with olive mill wastewater and agricultural wastes. Waste Manage. 28:2593-603.

Joshi AA, Mudappa D, Raman TRS, 2009. Brewing trouble: coffee invasion in relation to edges and forest structure in tropical rainforest fragments of the Western Ghats, India. Biol. Invasions 11:2387-400.

Misra RV, Roy RN, Hiraoka H, 2003. On-farm composting methods. Land and Water Discussion Paper, vol. 2. FAO Publ., Roma, Italy.

Niaounakis M, Halvadakis CP, 2006. Olive processing waste management: literature review and patent survey, 2nd ed. Elsevier Ltd. Ed., Kidlington, Oxford, UK.

Paredes C, Bernal MP, Cegarra J, Roig A, 2002. Bio-degradation of olive mill wastewater sludge by its co-composting with agricultural wastes. Bioresource Technol. 85:1-8.

Saviozzi A, Bufalino P, Levi-Minzi R, Riffaldi R, 2002. Biochemical activities in a degraded soil restored by two amendments: a laboratory study. Biol. Fertil. Soils 35:96-101.

Sellami F, Jarboui R, Hachicha S, Medhioub K, Ammar E, 2008. Co-composting of oil exhausted olive-cake, poultry manure and industrial residues of agro-food activity for soil amendment. Bioresource Technol. 99:1177-88.

Singh CP, Amberger A, 1998. Organic acids and phosphorus solubilization in straw composted with rock phosphate. Bioresource Technol. 63:13-6

Sofo A, Palese AM, Casacchia T, Celano G, Ricciuti P, Curci M, Crecchio C, Xiloyannis C, 2010. Genetic, functional, and metabolic responses of soil microbiota in a sustainable olive orchard. Soil Sci. 175:81-8.

Violante P, 2000. Metodi di Analisi Chimica del Suolo - Ministero delle Politiche Agricole e Forestali. Franco Angeli Ed., Milano, Italy.

Zaitlin B, Turkington K, Parkinson D, Clayton G, 2004. Effects of tillage and inorganic fertilizers on culturable soil actinomycete communities and inhibition of fungi by specific actinomycetes. Appl. Soil Ecol. 26:53-62.