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Short-term modifications of soil microbial community structure and soluble organic matter chemical composition following amendment with different solid olive mill waste and their derived composts

Ermanno Federici[a,1], Luisa Massaccesi[b,1], Daniela Pezzolla[c,1], Laura Fidati[a], Elena Montalbani[a], Primo Proietti[b,*], Luigi Nasini[b], Luca Regni[b], Silvia Scargetta[a], Giovanni Gigliotti[c]

[a] Department of Chemistry, Biology and Biotecnology, University of Perugia, Via Elce di sotto, 8 Perugia, Italy
[b] Department of Agriculture, Food and Environmental Sciences, University of Perugia, Borgo XX Giugno, 74 Perugia, Italy
[c] Department of Civil and Environmental Engineering, University of Perugia, Via G. Duranti, 63 Perugia, Italy

A R T I C L E I N F O

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A B S T R A C T

Production and disposal of olive-mill wastes represent a problem of environmental relevance particularly in the Mediterranean countries where they are generated in huge quantities.

A field trial was carried out to evaluate the short-term effects of soil amendment with solid olive mill waste (OMW) and its compost in comparison to the olive mill waste derived from the new two-phase treatment (humid OMW) and its resulting compost. The impact of treatments on soil water extractable organic matter and soil microbiota was determined. During a 120 days-long field trial, it was observed an initial increase of water extractable organic carbon (WEOC), total reducing sugars and total phenol compounds for all the amended soils. Even if humid OMW had the highest WEOC and total phenol compounds concentration, it increased to a lesser extent their content in the soil, probably due to the rapid utilisation of this particular organic matter by microorganisms. After three days from spreading, all amendments induced rapid modifications of both fungal and bacterial communities. However, while the bacterial community restored its initial state at the end of the trial, suggesting their high resilience capacity, the fungal populations changes remained until 120 days. The overall results indicated that all types of the organic materials tested as soil amendments had an impact on the soil microbial communities, especially in the early days of the field trial, without negative effects on the soil chemical characteristics.

1. Introduction

In the Mediterranean countries, the disposal of olive mill wastes (OMW) represents a relevant environmental issue because they are produced in huge quantities in a relative short period of time corresponding to two-three months from olive harvesting (Mechri et al., 2011; Nasini et al., 2013). Italy is the only olive-oil producing country in the world where a legislation for disposal of OMW in soil exists. Italian Law n. 574/96 integrated with Legislative decree of 6th July 2005 allows the spreading of up to 50 or 80 $m^3\ ha^{-1}\ yr^{-1}$ olive-mill wastewater generated by press or continuous centrifugation systems, respectively (Altieri and Esposito, 2008). Traditional three-phase oil extraction systems produce two by-products, a liquid one commonly known as olive mill wastewater and a solid olive mill waste, often referred as olive press cake or traditional husk. On the contrary, the new two-phase technologies result in the production of only a viscous, sludge-like OMW, known as humid husk or named "alperujo" in Spain (Alfano et al., 2008; Ntougias et al., 2006). Both type of OMWs are characterized by an undesirable odour, acidic pH, abundant organic load and high concentration of salts and phenols (Roig et al., 2006), features that make these materials a possible threat for surface and ground water pollution (Kistner et al., 2004). Furthermore, the high moisture content of two-phase OMW reduces its economic value and their disposal has become a new problem for the oil extraction industry (Sánchez-Monedero et al., 2008). Therefore, several valorisation and recycling methods of these wastes have been proposed, such as land spreading that can be considered an interesting way for its operational simplicity and convenience as soil amendments (Altieri and Esposito, 2008; Gigliotti et al., 2012). Nevertheless, the direct use of OMW as a fertilizer may cause negative impacts on crops and the environment,

* Corresponding author at: Department of Agricultural, Food and Environmental Science, University of Perugia, Borgo XX Giugno 74, 06121, Perugia, Italy.
E-mail address: primo.proietti@unipg.it (P. Proietti).
[1] These authors contributed equally to the study.

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due to the presence of potentially phytotoxic and bacteriostatic substances (e.g. high content of polyphenols, organic acids and salts) (Roig et al., 2006).

In this context, different eco-friendly valorisation strategies, such as biological treatments to enhance OMW-degradation and detoxification by the implementation of advanced oxidation systems, anaerobic digestion and composting strategies, have been widely reported in the literature (Delgado-Moreno et al., 2007; Federici et al., 2009; Gigliotti et al., 2012; Plaza et al., 2007; Roig et al., 2006). The latter represents an interesting way to transform OMWs into non-leaching value-added products that can be used either as soil amendments in the field (Delgado-Moreno et al., 2009; Proietti et al., 2015) or as pot substrates in plant nurseries (Altieri et al., 2010; Del Buono et al., 2011). The application of organic amendments may modify the structure and function of soil microbial communities (Banerjee et al., 1997; Franco-Otero et al., 2012; Pezzolla et al., 2013), and have an effect on C, N and P cycles and, eventually, on soil fertility and crop production (Antolín et al., 2005; García-Ruiz et al., 2012; Gómez-Muñoz et al., 2012; Madejón et al., 2003). In particular, the application of organic amendments affects the autochthonous soil microbial biomass due to the introduction of exogenous populations of microorganisms and available organic compounds (Bastida et al., 2008; Enwall et al., 2007; Pezzolla et al., 2015).

Soil bacteria and fungi can maintain ecosystem functions and productivity by their contribution to a wide range of essential activities such as: decomposition processes and humic substances synthesis, C and nutrient cycling, enhancement of the uptake of nutrients and water (Lozano-García and Parras-Alcántara, 2013; Orgiazzi et al., 2012), protection of crops from pests and disease through biological control (Bonanomi et al., 2007; Pane et al., 2011) and bioremediation of toxic metals or other hazardous wastes (Delgado-Moreno and Peña, 2009; Gandolfi et al., 2010). In recent decades, a wide variety of molecular-based approaches to study microbial communities have been developed to overcome some of the limitations associated with traditional culture-based techniques. For example, it has been estimated that only ca. 1% of soil bacteria can be isolated and cultured using traditional methods (Federici et al., 2011; Kirk et al., 2004). Hence the introduction of molecular techniques has allowed much more detailed insights into the composition of microbial communities (Federici et al., 2012; Sampedro et al., 2009).

Furthermore, organic amendments are known to affect soil organic matter (SOM) particularly the quantity and the composition of dissolved organic matter (Chantigny, 2003; Gigliotti et al., 1997; Kalbitz, 2001). SOM is universally recognized to be among the most important factors, responsible for soil fertility and land protection from contamination, degradation, erosion and desertification, especially in semiarid areas (Senesi et al., 2007). Some previous studies show that SOM evolution also depends on the origin and chemical composition of the organic amendments (González-Ubierna et al., 2012). In particular the SOM fraction extractable by water, named as water extractable organic matter (WEOM) represents the most active and mobile form of SOM (Said-Pullicino et al., 2007). The importance of WEOM properties as indicators of change in soil quality has received great attention (Chantigny, 2003; Corvasce et al., 2006). In fact, several recent studies have suggested a vital role of WEOM in numerous biogeochemical processes, due to its ability to bind and transport anthropogenic materials (Businelli et al., 1999; Gigliotti et al., 2005; Kalbitz et al., 2000). In addition, WEOM is the main energy source for soil microorganisms, and it influences the availability of metal ions in soil by forming soluble complexes (Zhang et al., 2006). The study of the water extractable organic carbon (WEOC) in the fresh and composted OMW, derived from traditional three-phase systems and two-phase olive oil extraction technologies, before and after soil spreading, can be attractive from ecological and environmental points of view.

In this context, our previous studies (Nasini et al., 2013; Proietti et al., 2015; Regni et al., 2017) evaluated the effects of soil amendment with OMWs through long-term field trials (4 years). It was demonstrated that different kind of OMWs can be used, both as such and after composting, as soil amendment in olive orchards increasing the vegetative and productive activities of the trees, without long-term negative consequences on chemical and microbiological soil characteristics and retaining the same olive oil quality. Based on these findings, and on the hypothesis that these organic amendments could be only a short-term substrate for the soil microbiota, we investigated the short-term effects of soil amendment with OMWs. The aim of the present work was to study, during a 120-days field trial, the modifications of soil WEOM composition and the effects on soil microbiota induced by a humid husk, obtained from a new generation two-phase oil extraction plant, spread in an olive orchard either as a fresh and composted amendments, compared to fresh and composted traditional three-phase husk. Specifically, we tested the following hypotheses: (i) the chemical properties of the soil WEOM are influenced by the different treatment and, in particular, the composted materials improve the WEOC quality respect to the fresh materials; (ii) the soil microbial community structure and abundance is more affected by the humid OMW than the other treatments due to the high content of WEOC.

2. Materials and methods

A ten-year-old non-irrigated olive grove, with trees of the Leccino cultivar, located in central Italy near Assisi (12°56′E longitude, 43°11′N latitude, about 400 m a.s.l.) was used for this experiment. The soil, derived from calcareous marl and classified as Typic Haploxerept (Soil Survey Staff, 2010), was described in details in Nasini et al. (2013).

2.1. Organic materials and soil amendment and sampling

The organic materials used in the experiment were: solid olive mill waste (OMW) and its compost (compost OMW) compared to the olive mill waste derived from the new two-phase treatment (humid OMW) and its resulting compost (compost humid OMW). The two composts were obtained as described in Gigliotti et al. (2012). Any organic amendment matrices were spread (30 kg d.w. for tree) in three plots (9 olive tree each) for a total of 12 plots. Other three non-treated plots served as the control. The soil was cover cropped and, thus, the organic amendments were not incorporated. Soil samples, after amendment residues removal, were periodically collected from the Ap horizon (0–15 cm of depth) during the following 4 months (at 3, 15, 30, 60, 120 days) after the land spreading. One soil sample for each plot (15 for each sampling time) was collected for chemical analyses, whereas for microbiological analyses the soil samples from the same treatment were pooled together.

The soil samples collected for the chemical analyses were air-dried and sieved through at 2-mm mesh, whereas the soil samples used for microbiological analyses were sieved at 2 mm at field moisture, stored in plastic bags and refrigerated at −20 °C.

2.2. Chemical analysis of OMW

Air-dried OMW and derived compost samples sieved at 2 mm were used for the chemical characterisation of the materials. Electrical conductivity (EC) and pH in water were determined in a 1:10 OMW: water extract ratio. Total organic C was analysed by an Elemental Analyser (EA1110 Carlo Erba, Milan Italy; ANPA, 2001), while total Kjeldahl-N (TKN) was determined by means of Kjeldahl distillation (ANPA, 2001).

Water-extractable organic matter (WEOM) was obtained as described by Said-Pullicino et al. (2007b). Briefly, OMW samples were extracted with deionized water (solid to water ratio of 1:10 w/w) for 24 h in a horizontal shaker at room temperature. The suspensions were then centrifuged at 10,000 rpm for 10 min and filtered through a 0.45 μm membrane filter. The water extracts were analysed for organic C using Pt-catalysed, high-temperature combustion (680 °C) followed