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Soil Biology & Biochemistry 40 (2008) 152–161

Soil Biology & Biochemistry

www.elsevier.com/locate/soilbio

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

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Received 10 February 2007; received in revised form 12 July 2007; accepted 19 July 2007
Available online 24 August 2007

Abstract

Olive mill wastewater (OMW) constitutes a major environmental problem for Mediterranean countries, where most of the world olive oil production takes place. The recycling of the OMW and its use as water for irrigation in agriculture, provided that its impact on soil and plant is established, is an attractive possibility for the Mediterranean countries. Investigations were performed on the influence of agronomic application of OMW (amount applied: 30, 60, 100 and 150 $m^3\,ha^{-1}$) in a field of olive trees on trees characters (photosynthesis, root-soluble carbohydrate and root colonisation), soil properties, and soil microbial community structure. Specific attention was paid to arbuscular mycorrhizal (AM) fungi. The soil fatty acid methyl ester (FAME) 16:1ω5 was used to quantify biomass of AM fungi and the root FAME 16:1ω5 analysis was used as index for the development of colonisation in the olive trees roots. A significant increase in organic C, C/N ratio, extractable phosphorus and exchangeable potassium was found after one year of agronomic application of OMW. The development of saprophytic fungi was significantly higher in the OMW amended soils, whereas the abundance of the soil FAME 16:1ω5, root FAME 16:1ω5, photosynthetic rates and the amount of the total root-soluble carbohydrate were decreased significantly after agronomic application of OMW. A principal component analysis (PCA) of the trees characteristics profiles showed discrimination between the nonirrigated and the OMW irrigated olive trees. These findings suggest that the altering functioning of arbuscular mycorrhizas should be considered as potential factors mediating olive trees responses to agronomic application of OMW when the OMW dose applied is higher than 30 $m^3\,ha^{-1}$. To our knowledge, this is the first report of alterations in the soil FAME 16:1ω5 and root FAME 16:1ω5 due to land spreading of OMW.

Keywords: Microbial community; Olive mill wastewaters; Arbuscular mycorrhizal fungi; Roots colonisation; Soluble carbohydrate

1. Introduction

The industrial olive oil sector generates large quantities of solid and liquid wastes and by-products in many Mediterranean countries during November–February. The chemical composition of the olive mill wastewater (OMW) is very variable. It depends upon the variety of the olive trees, fruit maturity, harvest time and processing method; the main characteristics are high values of chemical oxygen demand (COD) (60 000–185 000 $mg\,l^{-1}$) and biochemical oxygen demand (BOD) (14 000–75 000 $mg\,l^{-1}$), low pH, high polyphenols and high potassium content (Rinaldi et al., 2003). The gradual accumulation or incorrect disposal of these wastes may cause environmental problems. These materials must be treated or re-used if

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0038-0717/$ - see front matter
doi:10.1016/j.soilbio.2007.07.020

their environmental impact is to be reduced, enabling at the same time some of their primary components to be recovered (organic matter, nutrients, etc.).

Different disposal methods based on evaporation ponds, thermal concentration, physico-chemical and biological treatments have been used to deal with the major problem of organic load reduction (Rozzi and Malpei, 1996). These methods, however, are too expensive for wide application and do not solve the problem completely because of the need to dispose of sludge or other by-products derived from the process. For these reasons, increasing attention has been given to find the best methods to spread OMW on agricultural lands and to recycle both the organic matter and the nutritive elements in the soil crop system.

Soils in semiarid areas have a very low microbial activity, low levels of microbial biomass, low nutrient availability and a low organic matter content (García et al., 1994). OMW is rich in organic matter and an important source of nutrients for plants (Paredes et al., 1999; Rinaldi et al., 2003). Incorporation of organic materials, such as OMW, into soil can promote microbiological activity. Microbial activity and soil fertility are generally closely related because it is through the biomass that the mineralisation of the important organic elements (C,N, P and S) occur (Frankenberger and Dick, 1983). Paredes et al. (1999) found that OMW is known to increase soil organic matter and the concentration of essential inorganic elements for plant growth. Piotrowska et al. (2005), Cabrera et al. (1996) and Zenjari and Nejmeddine (2001) observed a temporary decrease in soil pH, increased salinity and elevated phenol concentrations following agronomic application of OMW. Negative effects of OMW on soil properties have also been recorded, including the immobilisation of available N (Saviozzi et al., 1991). Tardioli et al. (1997) mention that the addition of fresh OMW to soil increases the number of soil microorganisms (bacteria, yeasts and fungi), and induces a change in the microbial community.

Olive plants are known to form arbuscular mycorrhizal (AM) fungi (Calvente et al., 2004). There has been no investigation on the effects of the OMW on AM fungi, an important biotic component of agricultural soils. AM fungi are essential components of sustainable soil–plant systems (Van der Heijden et al., 1998). They play an essential role in enhancing plant growth in semiarid agro-ecosystem (McGee, 1989), supporting plants under biotic (e.g. pathogen infection) or abiotic stress (e.g. nutrient or water deficiency). AM fungi interface directly with the soil by producing extraradical hyphae that may extend several centimetres out into the soil (Rhodes and Gerdemann, 1975). Extraradical hyphae can have a total surface area of several orders of magnitude greater than that of roots alone, which increases the potential for nutrient uptake (Smith and Read, 1997; Hodge et al., 2001; Read and Perez-Moreno, 2003) and possibly also water uptake (Puppi and Bras, 1990). AM symbiosis can also alleviate negative effects of plant pathogens (Niemira et al., 1996; St-Arnaud et al., 1997) and toxic levels of metals (Khan et al., 2000). In addition, the extraradical hyphae may interact with other soil organisms either indirectly by changing host plant physiology, including root physiology and patterns of exudation into the mycorrhizosphere, or directly by physically and/or metabolically interacting with other organisms in the mycorrhizosphere. It has been estimated that in natural ecosystems plants colonised with AM may invest 10–20% of the photosynthetically fixed carbon in their fungal partners (Johnson et al., 2002). Clearly, this represents a significant input of energy into the soil ecosystem and this carbon may be crucial to microorganisms associated with the mycorrhizosphere.

Exchange of nutrients—mineral nutrients supplied by the fungal microsymbiont versus carbohydrates provided by the plant—is considered to be the main benefit for the symbiotic partners (Smith and Read, 1997). Positive correlations between carbohydrate availability in roots and AM formation have been reported (Graham et al., 1997). However, there are exceptions which include studies where no correlation (Amijee et al., 1993) or a negative correlation (Pearson and Schweiger, 1993) has been found between AM colonisation levels and root-soluble carbohydrates.

The analysis of fatty acids extracted from soil has been usefully used to study soil microbial community changes in agricultural soils (Bossio et al., 1998; Calderon et al., 2001). The soil fatty acid signature 16:1ω5 has been used to quantify biomass of AM fungi (Olsson et al., 1995). This fatty acid accumulates in roots during AM fungus colonisation (Graham et al., 1996; Olsson et al., 1995) and the amount accumulated is correlated to microscopically estimated measures of total root colonisation (Olsson et al., 1997). Fatty acid methyl ester (FAME) 16:1ω5 analysis of roots not only provided a measure of colonisation development but also served as an index of carbon allocated to intraradical fungal growth and lipid storage (Graham et al., 1995).

The association between plants and AM fungi has been defined as a mutualistic symbiosis, because usually both partners increase their fitness as a result of their interaction (Read, 1999). However, there is wide variation in the effectiveness of AM fungi on plant physiology and performance (Abbott and Robson, 1977, 1978), and the combination of host, fungi and soil conditions influence the relative costs and benefits of the symbiosis (Van der Heijden et al., 1998). There are also specific environmental factors that can shift the balance from beneficial to detrimental interactions. The following question was addressed in the present study: is the specific environment created after agronomic application of OMW beneficial or detrimental to soil and olive trees?

We hypothesized that OMW will increase soil organic matter and the concentration of essential inorganic elements in olive trees. The presence of these organic and inorganic substances in the soil is associated with a general increase in nutrient contents, with subsequent effects on