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Linking microbial functional diversity of olive rhizosphere soil to management systems in commercial orchards in southern Spain

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ABSTRACT

A comparative study of organic and conventional commercial olive farming systems was conducted in a wide-area of Andalusia, southern Spain to determine the effect of management practices on chemical and biological soil properties. Soils from 41 accredited organic farms, 49 conventionally managed neighboring farms, and three sites containing wild or feral forms of olive were analyzed. A polyphasic approach was used combining traditional soil physicochemical analysis, culture-dependent microbiological analyses, sole-carbon-source utilization profiles using the Biolog EcoPlate assay and enzymatic activities using the API ZYM assay. Different multivariate statistical analyses clearly demonstrated that olive orchard soils could be differentiated by farm management system. Discriminant analysis differentiated among three well defined soil groups that correlated with the farm management system (conventional, organic or wild olives). Thus, enzymatic activities and sole-carbon-source utilization profiles correctly classified 75, 77 and 100% of soil samples from orchards under organic and conventional management or wild olives, respectively. Overall, all diversity and functional indexes estimated from the Biolog EcoPlate and API ZYM assays were positively and significantly correlated with SOM, organic C and N content and the C:N ratio, and negatively and significantly correlated with clay content. In general olive orchards under organic management showed significantly higher SOM, organic C and N content, and C:N ratio, as well as higher microbial diversity as measured by catabolic capability and functional indexes of the Biolog EcoPlate and API ZYM assays as compared to conventionally managed orchards. Given the crucial importance of maintaining or increasing soil health in agricultural ecosystems, this is the first demonstration that commercial olive orchards under organic management in southern Spain are agricultural systems that may contribute to promote and conserve soil quality and health.

1. Introduction

The cultivated olive (*Olea europaea* L. subsp. *europaea* var. *europaea*) has been culturally and economically the main oleaginous crop in the Mediterranean Basin, where approximately 9.5 million ha of olives are grown, accounting for 95% of the cultivated olive area worldwide (Aranda et al., 2011; CAP, 2011; IOC, 2011). Spain is the world's largest olive oil producer, accounting for more than one-third of global production (CAP, 2011; IOC, 2011). In Andalusia (southern Spain), olive orchards dominate the landscape in an impressive monoculture that covers approximately 17% of the total surface of the region (1.5 million ha) (CAP, 2011; Soriano et al., in press).

During the past two decades, several major technological innovations have occurred that lower environmental impact of olive production in Spain. For example, soil management systems alternative to tillage, such as the use of cover crops, have been introduced to minimize soil erosion, a major problem of olive soils (Álvarez et al., 2007; Castro et al., 2008; Gómez et al., 2009b; Soriano et al., in press). In addition, new management systems such as integrated production and organic farming have been promoted to reduce the excessive use of chemical pesticides and inorganic fertilizers (Álvarez et al., 2007; Milgroom et al., 2007; Soriano et al., in press).

The use of these environmentally friendly agricultural practices in organic and integrated production systems, mainly through a reduction of tillage or use of cover crops, have proven to be highly effective in restoring and improving soil quality and soil health in Mediterranean-type environments as compared to conventional and intensive management systems (Álvarez et al., 2007; Bastida et al., 2012; Castro et al., 2008; Gómez et al., 2009a,b; Gomiero et al., 2011; Moreno et al., 2009; Soriano et al., in press). Nevertheless,

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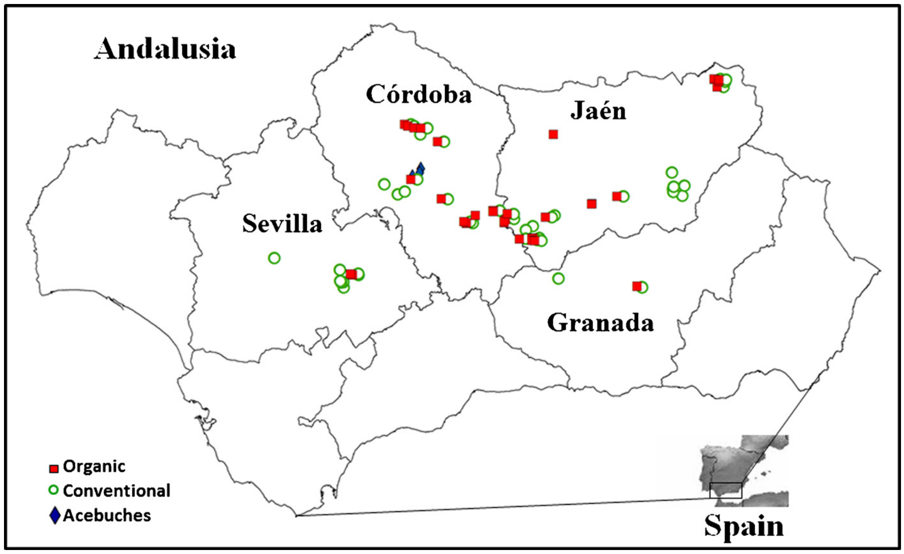

Fig. 1. Location of the 90 commercial olive orchards and 3 wild olive ('Acebuches') havens sampled in the provinces of Córdoba, Granada, Jaén, and Sevilla at Andalusia, southern Spain.

according to our knowledge, no wide-region studies have been conducted in Spain in commercial olive orchards to determine the effect of olive farm management systems on soil biological quality.

Evaluation of agricultural soil biological quality can be challenging (Bastida et al., 2008) because soil functions are affected not only by soil properties, but also by the complex interactions between climate, landscape, and management. Measurement of soil enzyme activities and/or microbial carbon source assimilation profiles have been shown to be appropriate methods for assessing changes in soil quality, soil recovery from disturbance or stress, and good indicators of microbial function because of their central role in nutrient cycling and their sensitivity to changes in management practices (Bastida et al., 2012; Benítez et al., 2006; Burns and Dick, 2002; Caldwell, 2005; Dick, 1992, 1994; García-Ruíz et al., 2009).

The objective of our study was to analyze the soil-microbial diversity in conventionally managed and organic commercial olive orchards and in sites containing wild or feral forms of olives in southern Spain. We measured microbial community structure and function through the use of sole-carbon-source utilization profiles and enzymatic activities using two assays, establishing the correlations between those factors with culturable bacterial population densities and soil physicochemical properties.

2. Materials and methods

2.1. Sites description and soil sampling

Soil samples were collected from 90 commercial orchards differing in management system [conventional (C, 49 orchards) vs. organic (O, 41 orchards)] located in the main olive-growing areas of Córdoba (41 orchards), Granada (3 orchards), Jaén (34 orchards), and Sevilla (12 orchards) provinces in Andalusia, southern Spain (Fig. 1 and Table 1). Criteria to select an orchard for sampling were: olive surface > 2 ha, olive production for at least 10 years, and current management system used for at least 5 years. Organic orchards sampled were accredited through the Andalusian Committee of Organic Farming (CAAE, Junta de Andalucía) which provided the database used for selecting the organic orchards. When possible neighboring side by side conventional and organic orchards were sampled. Orchards across all locations sampled differ in climate, soil texture, soil management system (use of cover crops vs. bare soil), irrigation regime (rain-fed vs. drip-irrigated), presence of cattle and use of manure or not (Table 1). In addition, three samples from three sites in Córdoba province containing wild or feral forms of olive (WO) (i.e., secondary sexual derivatives of the cultivated clones or products of hybridization between cultivated trees and nearby oleasters) were included in the study (Aranda et al., 2011).

Samples of rhizosphere soil were collected with a shovel at 5–30 cm depth in the area of the canopy projection (close to the influence of olive roots; i.e., rhizosphere soil) as described previously (Aranda et al., 2011). Eight trees (about 2 kg of soil/tree) were chosen randomly at each location, at the end of spring to the beginning of summer 2009 when enzyme activities are higher (García-Ruíz et al., 2009). Care was taken to avoid sampling after drip irrigation to prevent differences in soil moisture. All samples from all trees were thoroughly mixed in a composite sample, air-dried, sieved (2–5-mm mesh size) prior to soil physicochemical analyses and kept at 5 °C until processing for API ZYM and Biolog EcoPlate assays (less than one week after sampling). All measurements described below were made on three soil sample replicates per olive orchard.

2.2. Processing of rhizosphere soil samples

Physicochemical properties of soils including soil texture, pH, organic carbon (O) and nitrogen (N) content, and cation exchange capacity (CEC) were determined by the official Agroalimentary Laboratory of Córdoba (Córdoba, Spain) as described in Álvarez et al. (2007) and Soriano et al. (in press). Rhizosphere soil suspensions were prepared by mixing 5 g of soil from each sample with 50 ml of sterile saline solution (0.85% NaCl, w/v). The soil suspension was shaken at 350 rpm for 60 min using an orbital shaker and centrifuged at 1000 × g for 3 min. This soil supernatant was used directly for enzymatic activity (API ZYM) and a 1/10 dilution of it in 0.85% NaCl for Biolog EcoPlate analysis.

2.3. Measuring enzymatic activities

Enzymatic activities in olive orchard soil samples were determined by the API ZYM (BioMerieux, Madrid, Spain) system. The API ZYM strips consist of 20 microcupules containing dehydrated chromogenic substrates of 19 different enzymes and a control (a microcupule that does not contain any enzyme substrate). These enzymes include three phosphatases (alkaline phosphatase, acid phosphatase and phosphohydrolase), three esterases (lipase, esterase–lipase and esterase), three aminopeptidases (leucine