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Functional and genetic fingerprinting of microbial soil community in a kiwifruit orchard under different management practices

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Soil quality depends on a large number of chemical, physical, biological and biochemical factors, and its characterization requires the selection of properties sensitive to management practices. Soil microbial community is a very susceptible index of soil quality and is of fundamental importance for ecosystem functioning, through determining nutrient cycling, organic matter decomposition and energy flow. However, there are so far few evidences whether and to what extent a different soil management determines changes on microbial community structure and activity in Mediterranean orchards. The aim of the present study was to explore two different agricultural management systems ('innovative' and 'conventional') on both activity and composition of soil microbial communities using a combination of physiological and molecular techniques. 'Innovative' management system included no-tillage, cover crops, guided fertilisation and irrigation, compost application, green pruning. Conventional management system included: soil tillage, chemical fertilisation, removal of pruning residues from the field, empirical criteria for irrigation and pruning.

The application of different soil management practices started in 2003 in a kiwifruit (*Actinidia deliciosa* A. Chev., cv. 'Hayward') orchard located in Bernalda (Matera, southern Italy). After four years, soil was sampled at depths of 0-10 cm and 10-20 cm. Analysed soil samples derived from 20 sub-samples (composite sample) and were collected according to a random scheme. Total DNA and RNA were extracted from

rhizospheric soil samples through a direct method (Fast Prep System; MP Biomedicals and MO BIO isolation kits, respectively). The extracted RNA was retro-transcripted by RETROscript Reverse Transcription for RT-PCR (Ambion). Soil DNA and c-DNA were amplified with two different sets of primers used to amplify a 500 bp region of the 16S r-DNA (Bacteria) and a 390 bp region of the 18S r-DNA (Fungi). PCR products were separated by DGGE in 6% polyacrylamide gel, 45%-60% urea–formamide (Bacteria) and 8% polyacrilamide gel, 30%-60% urea-formamide for (Fungi). Carbon source utilization patterns of soil microbial community (CLPPs) were assessed by using Biolog® 96 wells Eco-Microplates (Biolog Inc., USA) inoculated with 10^4 cells and incubated at 25 ˚C. Soil microorganisms were counted on TBA implemented with cycloheximide 0.1 mg/ml (Bacteria) and on MEA implemented with streptomycin 0.03 mg/ml and tetracycline 0.02 mg/ml (Fungi) after incubation for 72 h at 28˚C. Sybr Green I stained gels were recorded with a Bio-Rad Gel Doc 2000 Documentation system and DGGE banding patterns were analyzed by cluster analysis using the Gel Compare II software (Applied Maths) to generate dendrograms and Dice coefficients of similarity (UPGMA). Functional diversity from Biolog® data were evaluated by Shannon's substrate diversity index (H'), substrate richness (R) and substrate eveness (E).

Dendrograms obtained from DGGE analysis and values of substrate richness obtained from Biolog® analysis indicate that 'innovative' management system determined significant changes in soil microbic community. Our results suggest that the 'innovative' management system can be an efficacious method to increase soil chemical and biological fertility, enhance atmospheric CO_2 fixation and create optimal conditions for plant nutrition in semi-arid Mediterranean environments.