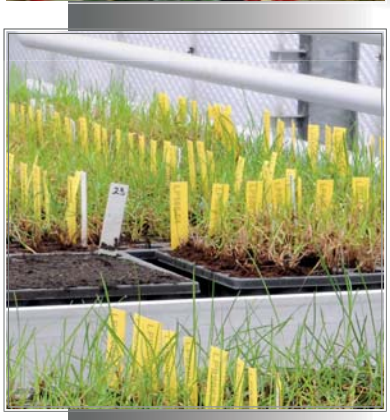

NUTRIHORT

Nutrient management, innovative techniques and nutrient legislation in intensive horticulture for an improved water quality

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(56) Soil microbial fertility in olive orchards managed by a set of sustainable agricultural practices

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Abstract: *In conventional olive growing, the frequent tillage has reduced soil organic matter content. Recently, soil conservation is becoming a priority in management strategies of rural areas due to the awareness of the deterioration of this natural resource and of the difficulty of its recovery in short periods (i.e. cross compliance concept in European Union). Therefore, the conventional agronomic practices should evolve to a more sustainable olive management to improve soil quality and water saving. A better understanding of soil ecology could lead to identify agricultural management practices that support and stimulate soil organisms for beneficial purposes in agriculture. The aim of this study was to evaluate the effects of sustainable practices (grass cover and pruning residues recycling) on soil quality in a Mediterranean olive orchard. The trials were carried out in a mature olive grove (Olea europaea L. – cv Maiatica) located in Basilicata Region (Southern Italy) and managed according to two different soil management systems: the sustainable treatment (ST) and the conventional treatment (CT). Soil microbiological quality in the two systems was monitored by both microbiological cultural-dependent and molecular methods. In the ST olive orchard, soil microbiota showed a higher complexity and metabolic diversity. The adoption of 'innovative', sustainable, agricultural practices had positive effects on soil microbiota and its biodiversity which can influence soil fertility and plant growth by increasing nutrients availability and turnover. The results of this study encourage the use of sustainable agricultural practices able to enhance soil fertility and promote good-quality fruit production without detrimental effects on water and soil resources.*

Keywords: *Biolog®; Fungal identification; Olea europaea L.; Soil enzyme activities; Sustainable soil management.*

Introduction

Suitable agricultural management practices, such as minimum tillage or no tillage, recycling of carbon sources internal to the fruit grove and adequate irrigation, fertilization and pruning management, are recommended to save water, restore soil organic matter, increase soil suppressiveness against plant pathogens, and reduce erosion and environmental pollution (Lal, 2004; Postma et al., 2008; Gomiero et al., 2011; Ding et al., 2013). Sustainable and innovative soil management systems in fruit growing can determine an optimal plant nutritional equilibrium, avoid nutrients accumulation in soils and leaching risks, improve irrigation efficiency, and prevent soil erosion and root asphyxia (Sofo et al., 2010; Sofo et al., 2011). Furthermore, the optimization and innovation of low-impact agricultural techniques have positive effects on both soils and crop yields as they increase microbial biomass, activity and complexity (Kushwaha et al., 2000; Widmer et al., 2006).

In semi-arid Mediterranean agricultural lands, a new approach in fruit orchard management is imposed by environmental emergencies, such as soil degradation and water shortage (Lal, 2004; Hochstrat et al., 2006). In these areas, the use of agronomical techniques aimed at improving or preserving soil quality, health and fertility is particularly recommended (Kushwaha and Singh, 2005; Govaerts et al., 2008). In olive groves, a positive influence of sustainable orchard management systems on soil biochemical and microbiological characteristics was observed (Hernández et al., 2005; Benitez et al., 2006; Moreno et al., 2009; Sofo et al., 2010).

Metabolic microbial community diversity in the structure of soil bacterial and fungal communities can be estimated by different methods and techniques (Gomiero et al., 2011). One of the most reliable and interesting is the Biolog® metabolic assay, based on the ability of microbial isolates to oxidize different carbon and nitrogen sources (Zak et al., 1994; Singh, 2009). The community-level physiological profiles (CLPPs), obtained by the Biolog® method, has a high discriminating power among microbial soil communities from various soil environments or subjected to various treatments (Calbrix et al., 2005; Gelsomino et al., 2006; Singh et al., 2006). It is also true that this important data should be necessarily interpreted and accompanied by the use of culture-dependent methods, in order to obtain the right characterization of the microorganisms tested, and by the determination of the activities of some soil enzymes, that are important markers of soil fertility status (Nannipieri et al., 2003).

On this basis, the aim of the present study was to explore the medium-term effects (12 years) of two different management systems (namely, sustainable and conventional) on the soil microbial functional and metabolic diversity of a mature olive orchard located in Southern Italy. Rather than the effect on soil microbiota of single sustainable

agronomic practices, a set of sustainable agricultural practices was considered. Finally, a new method in the assessment of fungal metabolic diversity was also tested and discussed.

Materials and Methods

The trial was carried out in a mature olive grove (cultivar Maiatica, a double aptitude variety) located in Southern Italy (Ferrandina, Basilicata Region, Italy; 40°29′N, 16°28′E) and gr
trained and planted at a distance of about 8×8 m. From 2000, the olive orchard was divided into two plots managed according to different soil management systems: the sustainable treatment (ST) and the conventional treatment (CT). The former was conducted by soil management techniques based on the recycling of polygenic carbon sources internal to the olive orchard. Particularly, ST was permanently covered by spontaneous weeds, mowed at least twice a year. Crop residues and pruning material, produced annually, were left on the ground as mulch. The CT was managed by continuous tillage (milling at 10 cm soil depth) in order to not have any weeds on the soil. Heavy pruning was carried out every two years. Pruned residues were removed from the olive orchard.

In October 2011, soil sampling was performed in both treatments (CT and ST). For each treatment, four composite samples of bulk soil were randomly collected in the inter-row area from the top soil layer (0-10 cm) and immediately stored in sterilized plastic pots at 4°C after removing visible crop residues. Each composite sample was formed by three seven-cm-diameter cores pooled on site within a 0.50 cm-radius. This sampling type was used to minimize spatial variability, as the experimental set up refers to a whole-field trial and not to single plots (Bacon and Hudson, 2001; Tian et al., 2004).

Three replicates of 5 g-sub-samples (dry weight equivalent) of each soil sample were suspended in 45 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 Ringer solution. Aliquots were plated in triplicate on 1/10 strength TSA (Tryptic Soy Agar) medium amended with 0.1 mg ml^{-1} cycloheximide for bacterial counting, and inoculated in MEA (Malt Extract Agar) medium containing 0.03 mg ml^{-1} streptomycin and tetracycline 0.02 mg ml^{-1} in triplicate for fungal counting. Counting took place after suitable incubation period (72 h for bacteria and 120 h for fungi) at 28°C.

Sole carbon source utilization patterns of soil microbial communities, also called community-level physiological profiles (CLPPs), were assessed using the Biolog® 96-well Eco-Microplates™ (AES Laboratoire, France), containing 31 different carbon sources, for bacteria, and using Biolog® FF MicroPlates™ (AES Laboratoire, France), containing 93 different carbon sources, for fungi. Data were analysed to determine metabolic diversity indices, including average well color development (AWCD, the mean of the blanked absorbance values for all the substrates, that provides a measure of total cultural bacterial activity), Shannon's substrate diversity index (*H'*), substrate evenness (*E*, equitability of activities across all utilized substrates) and substrate richness (*S*, the number of utilized substrates) (Zak et al., 1994).

Soil enzyme activities were measured on fresh soil samples. *β*-glucosidase activity was determined according to Eivazi and Tabatabai (1988) and the units expressed as µg *p*-nitrophenol h^{-1} g^{-1} soil. The dehydrogenase assay was performed according to the method of von Mersi and Schinner (1991) and the units expressed as µg triphenylformazan h^{-1} g^{-1} soil. Fluorescein diacetate (FDA) hydrolytic activity was determined according to Green et al. (2006) and units was expressed as µg tyrosine h^{-1} g^{-1} soil; protease activity was determined according to Geisseler et al. (2009) and the units expressed as µg tyrosine h^{-1} g^{-1} soil.

The statistical analysis of data was carried out using the data analysis software system STATISTICA, version 6.0 (StatSoft Inc.; Vigonza, PD, Italy). The mean values of bacterial and fungal microbial counts, Biolog® metabolic indices (AWCD, *H'*, *E* and *S*), and soil enzyme activities (four independent replicates for each treatment; $n = 4$) were separated according to Student's *t* test at $P \leq 0.05$.

Results and Discussion

Our data evidenced significant differences between the sustainable, high carbon input soil management system (ST) and the conventional, low carbon input system (CT).

The impact of sustainable orchard management was reflected in the microbial populations. Both total cultivable fungi and bacteria were significantly higher in ST (Table 1). Particularly, the total fungal number in ST was approximately 7.4-fold higher than that found in CT, whereas the total bacterial number was 3.6-fold higher. Soil fungi, more than bacteria, strongly responded to changes induced by the presence of spontaneous cover crops and the release of biomass and pruning residues on the soil (Borken et al., 2002; Peixoto et al., 2006). The high fungal number in ST is an

important benefit, making soil conditions more adequate for the development of crops. Fungi are able to colonize the rhizosphere and use root exudates as carbon source, they supply roots with easily assimilable nitrates and play a key role in the biological control of root pathogens and in the maintenance of soil health. Govaerts et al. (2008) reported that total bacterial count are generally higher when residues are retained than when they are removed and minimum tillage occurred.

Table 1 Total bacterial and fungal counts in soils sampled from the sustainable (ST) and conventional (CT) treatments. Values are means ± standard deviation (n = 4). Means with different letters are significantly different between the two treatments at $P \leq 0.05$, according to Student's t test.

	Fungi (CFU x 10^4 g^{-1} dry soil)	Bacteria (CFU x 10^6 g^{-1} dry soil)
ST	21.4 ± 11.8 a	35.6 ± 16.7 a
CT	2.9± 1.9 b	10.0 ± 2.6 b

Soil bacterial metabolic diversity indices estimated by Biolog® CLPP refer to the number, variety and variability of microorganisms in a given environment, including diversity within and between groups, and they are usually higher in sustainable than in conventional soils (Bucher and Lanyon, 2005; Govaerts et al., 2008). The increase in organic inputs due to cover crops presence and plant residues management can be an important discriminating element for microbial substrate utilization, according to Carrera et al. (2007). Furthermore, crop residues retention in the field and changes in soil organic matter can affect the metabolic diversity of the soil microbial communities evaluated by Biolog® CLPP (Bending et al., 2002; Govaerts et al., 2008).

Soil bacterial metabolic diversity indices are usually higher in sustainable than in conventional fields. In our experiment, the CLPP obtained by the Biolog® method was used to differentiate the soil fungal and bacterial populations of the two systems. For both bacteria and fungi, the analysis of Biolog® metabolic indices shows that total AWCD, H' and S were significantly affected by soil treatment, being higher in ST than in CT (Table 2). This indicates a higher microbial diversity and complexity due to sustainable practices. The values of E measured for fungi were not statistically different between ST and CT (Table 2). Considering that high values of E indicate the high microbial number of some groups of microorganisms, it appears that the long application of the conventional management did lead to a predominance of few groups of fungi or bacteria in the whole soil microbiota.

Indices of metabolic diversity do not necessarily reflect the composition of the bacterial communities as two communities can have similar values of metabolic diversity indices but utilize different substrates. In our case, Biolog® absorbance values identified that the AWCD values of all the principal classes of fungal and bacterial carbon substrates, were significantly higher in the ST, with only some exceptions (simple sugars, amino sugars, biogenic amines and nucleotides) for fungi. This result confirms the higher microbial metabolic activity in both the classes of microorganisms for almost all the substrates utilized.

Table 2 Fungal and bacterial metabolic diversity indices measured by Biolog® in soils sampled from the sustainable (ST) and conventional (CT) treatments. Values are means ± standard deviation (n = 4). Means with different letters are significantly different between the two treatments at $P \leq 0.05$, according to Student's t test. AWCD = average well color development; H' = Shannon's substrate diversity index; E = substrate evenness; S = substrate richness.

		AWCD	H'	E	S
Fungi	ST	0.75 ± 0.02 a	4.25 ± 0.03 a	2.48 ± 0.02 a	51.60 ± 1.52 a
	CT	0.51 ± 0.12 b	3.85 ± 0.20 b	2.41 ± 0.07 a	39.75 ± 3.86 b
Bacteria	ST	0.83 ± 0.09 a	3.20 ± 0.03 a	2.54 ± 0.07 a	18.40 ± 1.52 a
	CT	0.36± 0.07 b	2.97 ± 0.05 b	2.67 ± 0.10 a	13.00 ± 1.58 b

Biolog® FF MicroPlates™ have been efficiently used to assess carbohydrate use and assimilation and to determine metabolic profiling of soil fungi (Hobbie et al., 2003; Singh, 2009) but our study reports for the first time the utilization of these specific plates for determining the fungal catabolic profile using a procedure similar to that adopted for bacteria by Zak et al. (1994). This was possible because Biolog® FF plates, usually used for fungal identification, contain a

specific tetrazolium dye that can be metabolised by fungi but not by bacteria (Preston-Mafham et al., 2002). These latter were inhibited by the antiobiotics added to Biolog® FF plates, as explained in materials and methods section.

The degree of soil microbial activity is generally well correlated with the activity of key soil enzymes (Nannipieri et al., 2003). The extra-cellular soil enzyme *β*-glucosidase is of basic importance in the soil carbon cycle, as it catalyzes the last reaction of the catabolic pattern cellulose, hydrolyzing cellobiose in glucose and making it available to microorganisms. The activity of this enzyme was observed to be higher in soils subjected to sustainable agronomic practices, where biomass turnover is enhanced and soil mineralization is lowered, and it is a reliable index of a productive soil (Eivazi and Tabatabai, 1988). In this experiment, *β*-glucosidase activity was significantly higher in ST (Table 3). Dehydrogenases isoforms are common to most microorganisms, with a predominantly intracellular localization, and they are able to oxidize organic matter. In our case, dehydrogenase activity, even though higher in ST, did not differ statistically between the two treatments (Table 3) likely because these enzymes are good indicators of the vitality of fungal and bacterial populations more than their metabolic activity (von Mersi and Schinner, 1991). The hydrolysis of FDA, not different between the two orchard systems (Table 3), summarizes the hydrolytic activity of several enzymes and it is related to the hydrolytic activities of fungi and bacteria. In this sense, the activity of the FDA hydrolase represents an overall index of the potential for release of organic nutrients from organic matrices (Green et al., 2006). Proteases are a group of hydrolytic enzymes linked to the nitrogen cycle, and their function is to catalyze the hydrolysis of proteins, oligopeptides and dipeptides, until the release of ammonia. Like the glucosidases, they represent a useful index of the evolution of soil organic matter (Geisseler et al., 2009). Indeed, in this experiment, protease and *β*-glucosidase activity were strictly related, both showing an increase in the orchard managed with sustainable agronomic practices (Table 3).

Table 3 Soil enzyme activities in soils sampled from the sustainable (ST) and conventional (CT) treatments. Values are means ± standard deviation (n = 4). Means with different letters are significantly different between the two treatments at $P \leq 0.05$, according to Student's t test.

	β-glucosidase	Dehydrogenase	FDA hydrolase	Protease
	(Units g^{-1} soil)			
ST	187.0 ± 4.7 a	194.0 ± 19.9 a	4.4 ± 0.4 a	6.7 ± 1.5 a
CT	151.3 ± 0.6 b	163.9 ± 1.6 a	4.2 ± 0.2 a	3.1 ± 0.9 b

Interestingly, the observed improvement of soil microbial activity and diversity due to a 12-year sustainable management were similar to those observed in other fields cultivated organically with similar agronomic practices and for the same period (Nautiyal et al., 2010).

Conclusion

The results demonstrated that soil microorganisms significantly respond to a sustainable orchard management characterized by the medium-term application of endogenous sources (cover crops and pruning residues) of organic matter. The sustainable agronomic practices resulted in profound changes in the soil microbial community, that showed higher complexity and metabolic diversity. This study confirms the necessity to guide the farmers towards choices of soil management based on organic matter inputs to ameliorate soil functionality and agronomic productivity of Mediterranean orchards.

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References

Bacon, J.R., Hudson, G., 2001. A flexible methodology for the characterisation of soils: a case study of the heavy metal status of a site at Dornach. Science of the Total Environment 264, 153–162.

Bending, G.D., Turner, M.K., Jones, J.E., 2002. Interactions between crop residue and soil organic matter quality and the functional diversity of soil microbial communities. Soil Biology and Biochemistry 34, 1073–1082.

Benitez, E., Nogales, R., Campos, M., Ruano, F., 2006. Biochemical variability of olive-orchard soils under different management systems. Applied Soil Ecology 32, 221–231.

Borken, W., Muhs, A., Beese, F., 2002. Changes in microbial and soil properties following compost treatment of degraded temperate forest soils. Soil Biology and Biochemistry. 34, 403–412.

Bucher, A.E., Lanyon, L.E., 2005. Evaluating soil management with microbial community-level physiological profiles. Applied Soil Ecology 29, 59–71.

Calbrix, R., Laval, K., Barray, S., 2005. Analysis of the potential functional diversity of the bacterial community in soil: a reproducible procedure using sole-carbon-source utilization profiles. European Journal of Soil Biology. 41, 11–20.

Ding, G.C., Piceno, Y.M., Heuer, H., Weinert, N., Dohrmann, A.B., Carrillo, A., Andersen, G.L., Castellanos, T., Tebbe, C.C., Smalla, K., 2013. Changes of soil bacterial diversity as a consequence of agricultural land use in a semi-arid ecosystem. PLoS One 8, e59497.

Geisseler, D., Horwath, W.R., Doane T.A., 2009. Significance of organic nitrogen uptake from plant residues by soil microorganisms as affected by carbon end nitrogen availability. Soil Biology and Biochemistry 41, 1281–1288.

Gelsomino, A., Badalucco, L., Ambrosoli, R., Crecchio, C., Puglisi, E., Meli, S.M.., 2006. Changes in chemical and biological soil properties as induced by anthropogenic disturbance: A case study of an agricultural soil under recurrent flooding by wastewaters. Soil Biology and Biochemistry 38, 2069–2080.

Gomiero, T., Pimentel, D., Paoletti, M.G., 2011. Environmental impact of different agricultural management practices: conventional vs. organic agriculture. Critical Reviews in Plant Science 30, 95–124.

Govaerts, B., Mezzalama, M., Sayre, K.D., Crossa, J., Litcher, K., Troch, V., Vanherck, K., 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. Applied Soil Ecology 38, 197–210.

Green V.S., Stott D.E., Diack M., 2006. Assay for fluorescin diacetate hydrolytic activity; optimization for soil samples. Soil Biology and Biochemistry 38, 693–701.

Hernández, A.J., Lacasta, C., Pastor., J., 2005. Effects of different management practices on soil conservation and soil water in a rainfed olive orchard. Agricultural Water Management 77, 232–248.

Hochstrat, R., Wintgens, T., Melin, T., Jeffrey, P. 2006. Assessing the European wastewater reclamation and reuse potential – a scenario analysis. Desalination 188, 1–8.

Kushwaha, C.P., Singh, K.P., 2005. Crop productivity and soil fertility in a tropical dryland agro-ecosystem: impact of residue and tillage management. Experimental Agronomy 41, 39–50.

Kushwaha, C.P., Tripathi, S.K., Singh, K., 2000. Variations in soil microbial biomass and N availability due to residue and tillage management in a dryland rice agroecosystem. Soil and Tillage Research 56, 153–166.

Lal, R., 2004. Soil carbon sequestration to mitigate climate change. Geoderma 123, 1–22.

Moreno, B., Garcia-Rodriguez, S., Cañizares, R., Castro, J., Benítez, E., 2009. Rainfed olive farming in south-eastern Spain: Long-term effect of soil management on biological indicators of soil quality. Agriculture, Ecosystems and Environment 131, 333–339.

Nannipieri, P., Ascher, J., Ceccherini, M.T., Landi, L., Pietramellara, G., Renella, G.,2003. Microbial diversity and soil functions. European Journal of Soil Biology 54, 655–670.

Nautiyal, C.S., Chauhan, P.S., Bhatia, C.R., 2010. Changes in soil physico-chemical properties and microbial functional diversity due to 14 years of conversion of grassland to organic agriculture in semi-arid agroecosystem. Soil and Tillage Research 109, 55–60

Peixoto, R.S., Coutinho, H.L.C., Madari, B., Machado, P.L.O.A., Rumjanek, N.G., Van Elsas, J.D., Seldin, L., Rosado, A.S. 2006. Soil aggregation and bacterial community structure as affected by tillage and cover cropping in the Brazilian Cerrados. Soil and Tillage Research 90, 6–28.

Postma, J., Schilder, M.T., Bloem J., van Leeuwen-Haagsma,. W.K., 2008. Soil suppressiveness and functional diversity of the soil microflora in organic farming systems. Soil Biology and Biochemistry 40, 2394–2406.

Preston-Mafham, J., Boddy, L., Randerson, P.F., 2002. Analysis of microbial community functional diversity using sole-carbon-source utilisation profiles - a critique. FEMS Microbiology Ecology. 42: 1–14.

Singh, B.K., Munro, S., Reid, E., Ord, B., Potts, J.M., Paterson, E., Millard, P., 2006. Investigating microbial community structure in soils by physiological, biochemical and molecular fingerprinting methods. European Journal of Soil Science 57, 72–82.

Sofo, A., 2011. Drought stress tolerance and photoprotection in two varieties of olive tree. Acta Agriculturae Scandinavica - Section B 61, 711–720.

Sofo, A., Palese, A.M., 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 Science 175, 81–88.

Tian, G., Vose, J.M., Coleman, D.C., Geron, C.D., Walker, J.T., 2004. Evaluation of the effectiveness of riparian zone restoration in the southern Appalachians by assessing soil microbial populations. Applied Soil Ecology 26, 63–68.

von Mersì, W., Shinner, F., 1991. An improved and accurate method for determining the dehydrogenase activity of soils with iodonitrotetrazolium chloride. Biology and Fertility of Soils 11, 216–220.

Widmer, F., Rasche, F., Hartmann, M., Fliessbach. A., 2006. Community structures and substrate utilization of bacteria in soils from organic and conventional farming systems of the DOK long-term field experiment. Applied Soil Ecology 33, 294–307.

Zak, J.C., Willig, M.R., Moorhead, D.L., Wildman, H.G., 1994. Functional diversity of microbial communities: a quantitative approach. Soil Biology and Biochemisdtry 26, 1101–1108.